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POSSIBLE LONG TERM HEALTH CONSEQUENCES OF GULF WAR EXPOSURES: AN INDEPENDENT EVALUATION

INTRODUCTION

This chapter provides an independent examination of the long-term health consequences of Gulf War exposures by nationally recognized scientific experts. Chapter Three reviewed many of the complexities associated with the question of "Why are Gulf War veterans ill?" as well as some of the reasons why this question may never be answered. In an effort to examine what is known regarding the health effects of some of the exposures experienced by troops during the Gulf War, the SIU contracted with the following scientists.

This chapter contains the brief reports prepared by the consultants listed below. (The consultants' affiliations are provided for identification purposes only.) They are, in the order their reports appear in this chapter:

Fredric Gerr, M.D., Peachtree Environmental Consultants Inc., Decatur, Georgia; and Associate Professor, Department of Environmental and Occupational Health, Rollins School of Public Health of Emory University, Atlanta, Georgia. Dr. Gerr examined the chemicals that were in the Gulf, such as solvents, pesticides, depleted uranium, and others, for their potential health effects particularly upon the brain and nervous system. (Dr. Gerr's detailed report is at Appendix II.)

Matthew Keifer, M.D., M.P.H., Assistant Professor, Occupational and Environmental Medicine Program, Departments of Medicine and Environmental Health, Harborview Medical Center, University of Seattle, Washington. Dr. Keifer examined the total range of health effects to exposures to pesticides and related chemicals such as pyridostigmine bromide and some chemical nerve agents that are similar to pesticides. (Dr. Keifer's detailed report is at Appendix JJ.)

James Moss, Ph.D., Gainesville, Florida. Dr. Moss looked at the use of PB as it acts with combinations of other agents such as certain pesticides.

Richard Letz, Ph.D., Peachtree Environmental Consultants Inc., Decatur, Georgia; and Associate Professor, Department of Behavioral Sciences and Health Education, Rollins School of Public Health

of Emory University, Atlanta, Georgia. Dr. Letz evaluated the health effects of stress as an occupational and or environmental exposure in the Gulf.

Michael Lebowitz, Ph.D., Professor of Medicine, Pulmonary and Critical Care Medicine; Professor and Director of Epidemiology, Arizona Prevention Center; Chair, Epidemiology Graduate Interdisciplinary Program, University of Arizona, Tucson. Dr. Lebowitz examined the long-term health effects of sources of indoor and outdoor air pollutants during the Gulf War including oil well fires, sand, space heaters used in unvented tents, and other sources. (Dr. Lebowitz's detailed report is at Appendix KK.)

Kevin Dybvig, Ph.D., Professor, Departments of Comparative Medicine and Microbiology, University of Alabama at Birmingham. Dr. Dybvig evaluated the potential role of infection with *Mycoplasma fermantans* in the health problems of Gulf War veterans.

Shanna Swan, Ph.D., Chief, Reproductive Epidemiology Section, California Department of Health Services. Dr. Swan evaluated reproductive health issues from an epidemiological perspective. (Dr. Swan's detailed report is at Appendix LL.)

Melissa McDiarmid, M.D., M.P.H., Associate Professor of Medicine, Occupational Health Project, University of Maryland; and Director, Depleted Uranium Follow-up Program, Baltimore Veterans' Affairs Medical Center. Dr. McDiarmid examined the chemicals that were in the Gulf, such as solvents, pesticides, and depleted uranium, for their potential to adversely affect reproductive health outcomes. Dr. McDiarmid also examined the chemicals associated with the Gulf War deployment for their potential to increase the risk of cancer among Gulf War veterans. (Dr. McDiarmid's detailed reports are at Appendix MM and NN.)

HEALTH EFFECTS OF EXPOSURES TO NEUROTOXIC AGENTS USED IN THE PERSIAN GULF WAR

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SUMMARY

The purpose of this report is to review in detail the known health effects of chemical agents potentially hazardous to the nervous system to which military personnel may have been exposed during the Persian Gulf War. This review is made with special attention to possible relationships between these agents and symptoms and health complaints that have been reported by a large number of Persian Gulf War veterans.

On August 2, 1990, Iraq invaded Kuwait and set in motion the events that would eventually lead to US military intervention in the Persian Gulf. On August 8, 1990, the first US Air Force planes arrived in Saudi Arabia and, on the following day, the first US ground forces arrived. The ground war began and ended in February, 1991. The last of the US service members who served in the ground war were returned to the United States in June, 1991.

In all, the United States had approximately 697,000 troops stationed in the Persian Gulf. Following their return, mounting concern has focused on symptoms and unexplained illness experienced by some. In response to concern about unexplained illness, the VA Persian Gulf Health Registry was created. As of June, 1994, over 17,000 veterans, either ill or concerned about illness, had enrolled. The ten most frequent complaints among those in the Registry were fatigue (17.4%), rash (16.8%), headache (14.1%), muscle and or joint pain (13.9%), neuropsychologic complaints (10.5%), shortness of breath (7.5%), sleep disturbances (4.9%), gastrointestinal disturbance (4.1%), cough (3.8%), and other respiratory complaints (3.3%) (Persian Gulf Veterans Coordinating Board, 1995). The registry has not shed light on any distinctive demographic, exposure, or geographic risk factor, with the possible exception that nearly half of the veterans with symptoms were reservists/National Guard personnel, a group that accounted for only 17% of all troops deployed in the Persian Gulf (Persian Gulf Veterans Coordinating Board, 1995).

Numerous possible risks to health were present in the Persian Gulf at the time of the Gulf War. These included poor living conditions, characterized by heat and humidity, initially, and cold during the actual combat. Troops slept in temporary housing with little personal privacy. Food consisted

mainly of prepackaged meals. Flies and other insects were prevalent. Chemical warfare alarms sounded frequently, although virtually all were false. Such alarms, nevertheless, resulted in donning of air purifying masks and chemical protective clothing. Attention has been paid to possible chemical warfare agent exposure in the Gulf occurring as a result of destruction of a chemical warfare agent facility at Kamisiyah. Iraq was reported to have stockpiled biological warfare agents as well. Concern about health effects from exposure to these weapons as well as to indigenous infectious diseases lead to an extensive vaccination program. In addition, an estimated quarter of a million troops took the chemical warfare agent protective drug pyridostigmine bromide. Pesticides were used to control insect populations and insect repellents were provided to troops for personal use. Some troops were exposed to solvents from jet fuel, paint vapors, and other sources. Depleted uranium was used in special applications during the Gulf War and tetra-ethyl lead was formulated in gasoline used in motor vehicles. Finally, some troops were exposed to non-ionizing radiation from microwaves and radar installations (PAC, 1996).

In order to better characterize the health complaints of Gulf War veterans and to determine whether exposure to hazardous substances in the Gulf had caused them, health investigations of morbidity and mortality among Persian Gulf War veterans have been performed.

The largest and most methodologically sound study investigation included nearly five thousand subjects and involved inquiry about symptoms and exposure to known hazards in the Persian Gulf (Schwartz et al., 1997). Military personnel who served in the Persian Gulf War reported significantly more symptoms of depression, PTSD, chronic fatigue, cognitive dysfunction, bronchitis and asthma than non-Persian Gulf War personnel. Most of the self-reported exposures to hazards were statistically significantly related to virtually all of the health outcomes studied.

The results of the study indicate that subjective symptoms, including those consistent with nervous system impairment, occur more frequently among those who served in the Persian Gulf War than Persian Gulf War-era personnel who were not stationed in the Persian Gulf. The associations between multiple, unrelated exposures and multiple, unrelated symptoms, however, is more consistent with differential recall of exposure as a function of symptoms experience than a toxic response to a single or even several agents.

Several other studies intended to characterize with more objective measures the neurological health of Gulf War Veterans have been published. Authors of some suggest that the results show neither increased nervous system impairment nor a consistent pattern of illness suggestive of a common etiology (Amato et al., 1997; Jamal et al, 1996). Conversely, others conclude that their results show an increase in nervous system impairment and a pattern consistent with exposure to specific neurotoxicants (Haley et al., 1997). Unfortunately, nearly all of these studies were performed on "samples of convenience" and, as a result, cannot be used to draw conclusions about the larger but unstudied group of all Gulf War veterans. This body of literature has added little to the collective understanding of symptoms and health concerns among Persian Gulf War veterans.

Epidemiologic investigation of relationships between potentially toxic substances and ill health require accurate and unbiased assessment, on an individual basis, of both health status and the intensity and type of exposures experienced among a sample of persons representative of the entire group at risk. Of these requirements, the task that appears nearly impossible at this time is a person by person estimation of the intensity and type of exposures experienced by military personnel who served during the Persian Gulf War. Characterization of exposure to hazards was, apparently, not performed during the actual deployment of troops. As a result, estimation of the magnitude of past hazardous exposure at this time requires either direct questioning of veterans with resulting reporting bias or historical exposure reconstruction of unknown validity. As indicated above, reporting bias likely accounts for the associations observed in one study between symptoms and a very wide range of potential hazards.

As an alternative to epidemiologic investigation, another approach to investigating associations between health and hazardous exposure is to focus separately on 1) health problems among veterans and 2) exposures which they might have experienced. If a characteristic illness is observed among Gulf War veterans, then known causes for it can be explored. If particular hazards were encountered by veterans in the Gulf, the known health effects of exposure to them can be reviewed and compared to reported health problems among veterans. As neither approach attempts to relate exposure to illness on an individual basis, considerable caution must be exercised in their execution and interpretation. This report employs the latter of these two approaches and provides a systematic review of health effects of substances potentially toxic to the nervous system to which military personnel may have been exposed during the Persian Gulf War. A summary of the review is provided below.

Pyridostigmine bromide is an anticholinesterase drug given to tens of thousands of military personnel in the Persian Gulf war as a protective pre-treatment for exposure to “nerve gas” type chemical warfare agents (Dirnhuber et al, 1979). It is a member of the carbamate class of chemical agents and has been used for decades in humans as a treatment for the neurological disorder Myasthenia Gravis as well as a short acting accelerator of recovery from certain anesthetic agents. Pyridostigmine bromide acts by binding reversibly to, and consequently inhibiting, the enzyme acetylcholinesterase, which is necessary for normal function of the nervous system. This action is the basis for its ability to protect against the lethal effects of nerve agents which bind irreversibly to this enzyme. Pyridostigmine bromide is known to cause short-term discomfort and its use in the Gulf War was associated with abdominal distress, nausea, and diarrhea (Keeler et al., 1991; Sharabi et al., 1991). Little epidemiologic information is available about its long-term effects healthy young human populations, however, several factors suggest few or no long term effects on the nervous system. First, it has been used for decades for treatment of neurological illness with no systematic occurrence of symptoms resembling those experienced by Gulf War veterans. Second, the agent is not known to pass through the natural barrier that protect the brain from many drugs and chemicals (the “blood brain barrier”), thereby making effects on the brain unlikely. Third, the class of drugs and chemical

agents to which Pyridostigmine belongs, carbamates, have been used extensively in agriculture for decades and are not known to cause persistent adverse effects on the nervous system in that setting.

Chemical warfare agents, known as "nerve gas", are members of the organophosphate class of chemical compounds. The organophosphate nerve agents act to irreversibly bind the enzyme acetylcholinesterase (Grob and Harvey, 1957). Accumulation of the intended substrate of acetylcholinesterase, the neurotransmitter acetylcholine, results in a characteristic complex of symptoms. Unlike pyridostigmine, which also binds the enzyme acetylcholinesterase (reversibly, however), the organophosphate chemical warfare agents are capable of freely penetrating the brain and producing acute and chronic central nervous system toxicity.

Most of what is known about the effects of chemical warfare agents is a result of experimental studies of exposure to animals (Blick et al, 1994). However, several studies or case reports of acute human effects of exposure were identified in the literature (Grob and Harvey, 1957; Sidell, 1974). In addition, because of their chemical and toxicological similarity to organophosphate pesticides, some inferences about their toxicity can be made from the considerable literature about the organophosphate pesticides. Short term, acute exposure to chemical warfare agents produces a characteristic array of symptoms including sweating, diarrhea, urination, muscle twitching, pinpoint pupils, confusion, seizures, and, with sufficient exposure, death. Some credible medical evidence suggests that, upon recovery from toxic effects of acute exposure, chronic impairment of the central nervous system may occur (Sidell, 1974; Burchfiel and Duffy, 1982). Little evidence is available to suggest that exposures insufficient to produce acute toxicity are associated with long term neurological effects. Reportedly, no military personnel were treated for acute effects of nerve agent exposure, making unlikely that chronic effects of such exposure are the cause of symptoms experienced by Persian Gulf War veterans.

Organophosphate pesticides were used in the Persian Gulf for control of insects. Because of widespread use of organophosphate pesticides worldwide, a larger body of literature about the acute and chronic health effects of organophosphate pesticides on human populations, including chronic effects on the CNS, is available than is available for organophosphate chemical warfare agent agents.

In addition to the organophosphate class of pesticides, carbamate, pyrethroid, and organochlorine pesticides were also used. Only the organophosphate pesticides are known to cause, under certain exposure circumstances, long-term adverse effects on the nervous system. The carbamate pesticides, although similar in acute toxicity to organophosphates, are not known to result in long-term adverse neurological effects. Similarly, long-term adverse neurological effects of pyrethroid insecticides, and Lindane, the one organochlorine pesticide used in the Persian Gulf, have not been reported in the peer reviewed medical literature.

Exposure to organophosphate pesticides has been most convincingly associated with chronic adverse central nervous system health effects only when the exposure intensity is sufficient to

produce acute toxicity consistent with acetylcholinesterase inhibition (Steenland et al, 1994; Ames et al., 1995; Savage et al., 1988; Rosenstock et al., 1991). Only one report in the literature related exposures to levels of organophosphate pesticides insufficient to produce acute effects to long-term adverse effects on the central nervous system (Korsak and Sato, 1977). This finding has not been duplicated by other investigators. Given the apparent absence of documented signs and symptoms characteristic of acute organophosphate pesticide toxicity among soldiers deployed to the Persian Gulf, it unlikely that long-term health effects of pesticide toxicity is responsible for symptoms described by Persian Gulf veterans.

Lead, in the form of tetra-ethyl lead, was an octane boosting additive in gasoline used to fuel motor vehicles used by US forces in the Persian Gulf. Tetra-ethyl lead had been used in gasoline in the United States for decades and was widely discontinued from such use, for protection of the public health, beginning in the 1970's. Exposure to lead in the Persian Gulf War was limited to that emitted from vehicles in which leaded gasoline was used.

Both organic and inorganic lead are known to be toxic to the nervous system. Clinically, symptoms of lead intoxication include abdominal pain, fatigue, joint pain, headache, irritability and other mood disturbances, and muscle and joint pain. On clinical examination, physical signs of peripheral neuropathy, including paresthesias and motor weakness may be present (Culen et al., 1983). Clinical examination is insensitive to central nervous system impairment; however, when subjected to formal clinical neurobehavioral evaluation, patients with lead intoxication often show impairment of multiple central nervous system functions (Bordo et al., 1982; Baloh et al., 1980; Valciukas et al., 1978a. Valciukas et al., 1978b. Stollery et al., 1989; Hanninen et al., 1979; Mantere et al., 1984; Baker et al., 1985; Ashby, 1980).

Although leaded fuels were used in the Persian Gulf, it is unlikely that exposures to tailpipe emissions were of sufficient duration or intensity to produce any kind of clinically apparent toxicity from lead exposure. While long-term exposure to lead does result in accumulation of lead in long-term storage pools in the human body, short-term exposures result in little long-term accumulation. Failure of symptoms to remit for years following exposure is inconsistent with lead as an etiology of unexplained symptoms experienced by some Gulf War veterans. Furthermore, leaded fuels were used in the United States for decades and are still in use in many other countries worldwide. No reports of symptoms identical to those experienced by Persian Gulf veterans have emerged despite such widespread and long-term use.

Depleted uranium is a by-product of the extraction of uranium-235 (U235) from naturally occurring uranium. Military applications for this material include munitions production (armor piercing bullets and artillery shells) and armor for tanks and personnel carriers. The PGW was the first US use, in actual military conflict, of depleted uranium tipped shells and depleted uranium armored tanks and other vehicles (United States General Accounting Office, 1993).

At the current time, estimates of the total number of military personnel who had any exposure to depleted uranium are not available. Exposure may have occurred to personnel in vehicles penetrated by depleted uranium rounds as well as personnel involved in recovery and repair of vehicles damaged by depleted uranium containing rounds. The Army has identified 35 soldiers who were injured in combat vehicles damaged by depleted uranium munitions, 22 of whom likely were wounded by DU containing shrapnel. In addition, 27 soldiers involved in damage assessment and preparation for shipment of damaged combat vehicles have reported exposure to DU during those activities (United States General Accounting Office, 1993).

Exposure to uranium, depleted or non-depleted, is not known to produce adverse effects on the nervous system (Thun et al., 1985; Leggett, 1989; Morris and Meinhold, 1995). Reports of exposure to depleted uranium to soldiers in the Persian Gulf, although uncertain, suggest limited numbers of involved personnel. These facts make extremely unlikely that exposure to depleted uranium during the Gulf War is responsible, wholly or in part, for the array of symptoms observed among Gulf War veterans.

DEET, the common name for N,N-Diethyl-m-toluamide, is widely regarded as the most effective topical insect repellent available and is the major active ingredient in virtually all products marketed for this purpose (Robbins and Cherniack, 1986; Osimitz and Murphy, 1997). It was registered for use by the general public in 1957 and has been in civilian and military use since then. DEET has been a remarkably successful commercial product and is currently estimated to be used, in some form, by approximately 80 million persons in the United States, annually (Stinecipher and Shah, 1997). Despite relatively long-term use by millions, only a few reports of toxicity were found in the medical literature. Most descriptions of human toxicity come from case reports of individual exposures or from small case series. Among the 20 individuals described in case reports, the group most frequently affected by DEET exposure were children and the most commonly reported effects involved the nervous system (Osimitz and Murphy, 1997).

Several factors suggest that DEET is not responsible for the symptoms reported by some veterans of the Persian Gulf War. First, the product appears to have adverse effects only on a very small proportion of those who use it (Veltri et al., 1994). Second, the main adverse neurological effect appears to be seizures, a condition not reported commonly among Gulf War veterans, although one study of occupationally exposed workers has associated DEET with neurological symptoms with some similarity to those experienced by Gulf War veterans (as reported by Osimitz and Murphy, 1997 and Robbins and Cherniack, 1986). The symptoms were experienced at the time of exposure to DEET, however; no long-term follow-up was reported. All clinical studies of adverse effects of DEET suggest full recovery occurs after withdrawal of exposure. No literature is available to suggest that topical use of DEET results in long-term health consequences.

Solvents are simple organic substances that are (1) liquid at room temperature, (2) relatively non-reactive, and (3) able to dissolve a wide range of organic compounds (i.e., lipophilic). Most

solvents are quite volatile. The primary uses of solvents in the PGW were as motor vehicle and jet fuel, carriers for paint and coatings, and as an agent for control of airborne dusts blown from sand.

Solvents can affect the central nervous system (CNS), the peripheral nervous system (PNS), or both. Short term exposure to organic solvents can cause reversible anesthesia-like depression of the CNS. Long-term, heavy exposure to solvents may cause persistent, potentially irreversible impairment in cognitive function and affect, which may be associated with structural changes in neural tissue (NIOSH, 1987). Solvents can also cause impairment of peripheral nerve function (Spencer and Schaumburg, 1985).

Peripheral nervous system effects are well-established for a few specific solvents, none of which appear to have been used in the Persian Gulf (Spencer and Schaumburg, 1985). Acute, reversible CNS effects (i.e., acute intoxication) are common with all solvents (Laine and Riihimäki, 1986). Chronic, apparently fixed, adverse effects of solvents on the CNS have been reported in the literature, with general agreement that long-term occupational exposure to solvents is associated with adverse effects on multiple CNS domains and that persons who suffer from such effects may report symptoms similar to those reported by some Persian Gulf War veterans, including depression, impaired concentration, and memory loss (Hanninen, 1986; Danish Ministry of the Environment, 1991; Hogstedt, 1994; Spurgeon et al., 1992; Rasmussen et al., 1993; White et al., 1995; Daniell et al., 1993; Hänninen et al., 1991). The duration and intensity of exposure required to cause such effects and the potential severity of such effects is somewhat controversial, although most authorities agree that at least ten years of occupational (daily or near daily) exposure is required before effects are seen (Mikkelsen et al., 1988). Exposures to organic solvents in the Persian Gulf appear to be of insufficient duration, and may also have been of insufficient intensity, to produce chronic adverse effects on the CNS.

In summary, multiple agents with potential toxicity to the nervous system were used by military personnel in the Persian Gulf War. Such agents include pyridostigmine bromide, chemical warfare agents ("nerve gas"), pesticides, heavy metals, DEET, and organic solvents. Each of these agents or class of agents has been associated, in the biomedical literature, with acute or chronic toxicity to the central or peripheral nervous systems.

Soldiers returning from the Persian Gulf have reported numerous symptoms compatible with nervous system dysfunction including fatigue, headache, sleep disturbance, depression and memory impairment.

The concurrence of exposures with potential toxicity to the nervous system and the reporting of symptoms compatible with nervous system toxicity has lead to considerable scrutiny of a possible causal association between them. Review of the biomedical literature suggests, at this time, that neurotoxicity from exposure to pyridostigmine bromide, chemical warfare agents ("nerve gas"), pesticides, heavy metals, DEET, and organic solvents is not a likely explanation for symptoms

experienced by Persian Gulf War veterans. Reasons for this conclusion vary for each individual agent or class of agents but include insufficient duration of exposure, evidence of insufficient intensity of exposure, incompatibility of effects of exposure with symptoms reported by military personnel, and the chronicity of illness following removal from exposure.

While currently available evidence does not support a neurotoxicological etiology for symptoms reported by many Persian Gulf War veterans, some key issues remain unclear. To close these gaps in knowledge, the following recommendations are made:

To better characterize the neurological health status of Persian Gulf War veterans, a large study of a randomly selected sample of Persian Gulf War veterans and Persian Gulf War era veterans who did not serve in the Gulf in which objective measures of neurological and neurobehavioral function are used to assess neurological health should be performed.

Because clinical experience among healthy adults is limited, additional investigation of the long-term human health effects of pyridostigmine bromide in among healthy adults should be performed. Should pyridostigmine bromide be used by the US military in future conflicts, accurate records should be kept to permit fruitful long-term assessment of dose-effect relationships.

To determine whether exposure to pyridostigmine bromide altered military personnel responses to stress, investigation of the effect of pyridostigmine on physical and psychological responses to perceived threat of physical harm should be performed.

Because exposure to hazards rarely occurs in isolation, investigation of the effects of combined exposure to potentially toxic agents used in the Persian Gulf War should be performed. While such investigations may necessarily be performed on animals, the exposures used should be similar in route of administration, intensity, and duration to those experienced by humans under actual exposure conditions.

In the future, better efforts should be made to characterize objectively both health and hazardous exposures among US military personnel facing hazardous duty. Standardized, objective neurological and neurobehavioral testing of military personnel before deployment would provide useful baseline information about health status to which results of repeat testing, following deployment, could be compared. Quantitative assessment of exposure to potential hazards would provide information to compare to changes in health status that might be detected. The feasibility of such an effort should be explored.

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PERSISTENT HEALTH EFFECTS OF PESTICIDES AND OTHER CHEMICALS USED IN DESERT STORM AND DESERT SHIELD

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This report reviews the classes of pesticides, nerve gas, and prophylactic medication (pyridostigmine bromide) to which the Gulf War (GW) personnel were exposed, or potentially exposed, for the possibility that such exposure might be responsible for the chronic health problems known collectively as the Gulf War Syndrome. Recommendations for future research are also included.

Several different types of pesticides were imported to the Persian Gulf and acquired locally by American forces during Desert Storm and Desert Shield. While use patterns of neither imported nor locally acquired pesticides are documented, the quantities of imported pesticides are documented. Most of the imported pesticides were insecticides or repellents. Pesticides are by nature poisons most of which affect the nervous system. The potential for long term health effects resulting from exposure to many of these chemicals has been demonstrated in numerous studies and case reports with the nervous system being the principal focus of the majority of these reports.

The *oganophosphates*, a potent class of pesticides, appear to have been imported in large quantities. These chemicals have been clearly identified in many studies as a cause of both central and peripheral chronic neurological effects in persons who have sustained a heavy exposure (Keifer 1997, Rosenstock 1991, Steenland 1994, Savage 1988, McConnell 1994, Lotti 1986). It is important to note that nearly all cases of chronic neurological effects attributed to organophosphates resulted from overexposure which caused acute severe clinical illness. Most studies of subjects who have sustained less severe exposures or only chronic low level exposure have not observed these chronic neurological outcomes (Ames 1995, Fiedler 1997, Engel 1998).

One organophosphate, *chlorpyriphos*, which was shipped in large quantities (1580 gallons pure active ingredient, 3841 gallons of formulated product) and has been identified as capable of causing peripheral neuropathy in human beings following heavy exposure (Lotti 1986, Kaplan 1993), has recently come under careful scrutiny in the US because of its extremely broad use by both private citizens and pesticide applicators. The Health Effects Division of the Environmental Protection Agency reviewed the published literature and unpublished case reports and concluded that chlorpyriphos "may be a significant cause of chronic neurobehavioral effects". Unfortunately the report provided no exposure context in which these "chronic effects" might be expected to occur

(Blondell 1997). A recent study of morbidity by investigators from the manufacturer of chlorpyriphos identified an elevated risk for five diagnostic categories among its employees exposed to chlorpyriphos: 1. diseases of the ear and mastoid process; 2. acute respiratory infections; 3. other diseases of the respiratory system; 4. general symptoms, signs, and ill defined conditions; and 5. symptoms, signs and ill defined conditions involving the digestive system. (Burns et al. 1998). The illness categories identified by these investigators as showing higher rates in exposed workers reflect a broad assortment of signs and symptoms but of particular interest is the inclusion of the general symptoms category (numbers 4, ICD9 780-799).

The medical conditions included in this category are generally those that do not permit strict disease diagnosis by clinicians but interestingly this symptom category is the same as the third most common diagnosis identified by the Comprehensive Clinical Evaluation Program (CCEP) in evaluating 20,000 Persian Gulf veterans (Joseph et al. 1997). This overlap of diagnosis between workers exposed in an industrial setting and personnel exposed during the Gulf War experience potentially to the same chemical is intriguing. However, it should be pointed out that the situations are not directly comparable. How this chemical was used by personnel in the Gulf is not clearly documented (IOM 1996) where as exposure to the chemical is estimated in the Burns study. Additionally the workers who were reporting these illnesses through the company medical program were presumably actively exposed at the time of their reported illnesses and the CCEP study group was examined and questioned at time when presumably exposure to chlorpyriphos had ceased. Before conclusions that an excess prevalence of this diagnostic category in the CCEP study population is reached an adequate control population would be needed. There was no association drawn in either the EPA report or the morbidity study between chlorpyriphos and peripheral neuropathy, a condition affecting 0.2% of 20,000 veterans examined by the CCEP (Joseph et al 1997).

The other organophosphate pesticides included in the list of imported pesticides include one, *dichlorvos*, which has been identified in animal models as an inducer of peripheral neuropathy. However this chemical as used in the Gulf was enclosed in pest strips making significant overexposure less likely. No reports were found in the literature that environmental exposure to these pest strips caused significant illness or peripheral neuropathy.

The *N-methyl-carbamates* were imported in large quantities and while sharing the acute toxicological characteristics of organophosphates, have only rarely been associated with persistent health effects, and then only after chronic heavy exposure (Ecobichon et al 1982). The carbamates are in the same family of chemicals as pyridostigmine, the chemical used to prophylax personnel against nerve gas in the gulf. The pyrethroids, another category of pesticides, were brought over in large quantities, but are of relatively low acute toxicity and appear to be relatively safe pesticides (Aldridge 1990, He 1994).

Aluminum phosphide, a fumigant, was also imported in substantial quantities (20,020 tablets). These chemical tablets produce phosphine gas when combined with water. Phosphine is a very toxic gas which can produce severe illness in the setting of sufficient exposure. The illness produced by phosphine exposure would not be easily overlooked (Morgan 1989). Furthermore, based on how aluminum phosphide is generally used it is highly unlikely that low dose exposure to phosphine occurred. There is no evidence in the literature that chronic illness results from low dose exposure to phosphine.

In the absence of massive overexposure, each of these pesticides by itself, organophosphates, n-methyl-carbamates and pyrethroids, or phosphine, is not likely to have resulted in chronic health effects among even a substantial minority of U.S. troops.

Diethyl-m-toluamide (DEET) was imported in large quantities and presumably used widely as an insect repellent during the conflict (DOD on Aug 27, 1997 to Senator A. Specter). It is also widely used by the U.S. population in general and given its broad use (30 + % of the US population), the chemical has a reasonably good safety record (Veltri 1994). Case reports indicate that this chemical can induce central nervous system effects when absorbed in sufficient quantity but cases usually involve excessive exposure and often involve young children or infants. No reports in the literature describe the long term toxicity of DEET among humans with low level chronic exposure though some permanent residual effects have been noted in at least one case following recovery from what appeared to be an acute intoxication (Knowles 1992). The possibility that even relatively heavy exposure to DEET alone could induce chronic health effects in the Gulf personnel is unlikely.

Pyridostigmine bromide (PB), used by the U.S. forces as a prophylactic agent against the toxicity of nerve gas has demonstrable toxicity for both animal models and humans when given in relatively high dosage. The standard 30 mg three time per day dosage provided to U.S. forces may have caused acute toxicity in particularly susceptible populations such a asthmatics or soldiers with a unique serum cholinesterase phenotype (Loewenstein-Lichtenstein 1995), or in soldiers who received high per weight dosage because of small body mass (Gouge 1994) but this dosage has been shown to be generally well tolerated by the majority of the population (Blick 1994, Borland 1985, Cook 1992, Glikson 1991).

Studies on animals suggests that under stressful situations the lack of central nervous system penetration which makes PB an attractive prophylactic may not be assured. This central nervous system penetration may lead to acute central nervous system symptoms. Symptom persistence resulting from this increased penetration has not been reported to date in human or animal models, although evidence from one study presented indicated that a central nervous system feedback mechanism may account for changes which may outlast the acute cholinergic effects of the drug (Freidman et al 1996).

No information was found as to whether the *bromide* in the preparation might have had deleterious effects given bromide's long half-life and the desert conditions of chronic high heat and salt depletion. Despite these caveats, the years of experience in treating patients for myasthenia gravis with PB at doses often much higher than those taken by Gulf War service personnel would suggest that the development of persistent health effects among Gulf War personnel from PB alone is unlikely. The pyridostigmine is rapidly metabolized and the bromide is excreted over several weeks once the drug administration is stopped. The penetration of the blood brain barrier by pyridostigmine under the stress of a combat situation may potentially result in acute effects given sufficient blood levels, but with metabolism of the drug and the reversal of the acute effects, it is unlikely that long term effects would ensue.

The health effects of exposure to *nerve gases* has been only periodically addressed in the mainstream literature. One excellent study which examined most of the important nerve gases for production of peripheral neuropathy showed that sarin was capable only at super-lethal doses of potentially inducing neuropathy (Gordon et al. 1983). Few cases of known human exposure to nerve gases are available to examine for long term effects, so predictions must be modeled mostly from animal experiments. The Center for Disease Control concluded in 1988 that there appeared to be little risk of adverse health effects from low level long-term exposure to GA, GB, VX, H, HD, HT or lewisite (CDC 1988). In a review of the literature on nerve agents, Gunderson et al. concluded that persistent effects such as psychological and behavioral problems, could result after acute exposure, but that no evidence supported persistent effects from low level exposure to these chemicals (Gunderson et al.1992). A recently published study on survivors of the Japanese subway sarin gas incidents identifies possible delayed effects on balance among surviving female victims. These authors also cite an as yet unpublished manuscript identifying neurobehavioral abnormalities among other victims 6-8 months after the poisoning (Yokoyama et al. 1998). These findings are consistent with problems identified among persons previously poisoned with organophosphate pesticides (Keifer et al. 1997, Steenland et al.1994, Rosenstock et al. 1991, McConnell et al. 1994, Savage et al.1980, Lotti et al. 1986), which are related to the military nerve gases. The literature does not provide evidence to support persistent neurological or other health effects from low-level exposure to nerve gases.

From the information presently available, it does not appear that the DOD has a policy for *monitoring cholinesterase* or for assessing the physiological effects of the prescribed standard prophylactic dose of pyridostigmine bromide. The broad application of cholinesterase monitoring for all those taking PB doses would probably not be beneficial. Most people taking the drug would probably have a very predictable response to the dosage. The drug generally appears to be safe when taken by individuals of average size (70 kg), with normal uninhibited cholinesterase activity and with no illnesses which would make them particularly susceptible to ill effects from the PB. However, there is a substantial minority of individuals who may be smaller in stature, have illnesses such as asthma or, in rare cases, have congenitally low cholinesterase which makes them sensitive to PB even when taken in the prescribed dose. A mechanism should be in place to identify those who might

suffer ill effects and determine how their dosage should be adjusted in order to avoid complications while still providing protection from nerve gases.

Cholinesterase monitoring has long been used among pesticide applicators to identify overexposure to organophosphates. It also can potentially be used to identify personnel exposed to organophosphate nerve gas. Accurate interpretation requires a pre-exposure baseline on a subject against which to compare subsequent values. This limitation, and problems with the accuracy of commercially available test kits, makes cholinesterase testing complicated. Recently, a new approach to identifying overexposure to organophosphate nerve gas has been described. This method reactivates inhibited cholinesterase and reconstitutes the nerve gas molecule which can then be measured (Polhuijs et al. 1997). If this technique shows itself to be sound, it has potential application in determining whether personnel have sustained exposure to nerve gas even several weeks after exposure.

The potential for chronic health effects resulting from *mixtures of chemicals and from mixtures of pyridostigmine bromide and pesticides* is a subject of interest and recent investigation, though relatively little has been published to date. Studies on laboratory animals have demonstrated that in sufficient dosage, damage to the nerves of the body can occur with mixtures of some of the chemicals used by service personnel in the Gulf War conflict (Abou donia 1996a & b). An important caveat to these studies is that the dosages used to induce these damages were well above what would have been expected to occur by regular use of these chemicals. Studies of the effects of DEET on the absorption of pyrethroids and carbaryl (an n-methyl-carbamate) do not support the contention that more chemical is absorbed in the presence of DEET (Baynes et al 1997).

SUMMARY

A fair degree of uncertainty surrounds the exposures that may have occurred to personnel during Desert Shield and Desert Storm. Nevertheless, based on the information available in the literature regarding the pesticides and anti-personnel chemicals to which troops may have been exposed in the GW, chronic health effects would not be expected in any significant number due to low level exposure to these chemicals or to combinations of these chemicals. A small percentage of the population may have had reactions to these chemicals not predicted by animal research or human studies and given exposure sufficient to result in acute toxicity, chronic problems would not be surprising. Information sited in this report does raise questions about the possible non-specific symptoms reported by a substantial percentage of CCEP subjects and how this might relate to pesticide exposures which occurred in GW personnel. This relationship is uncertain but intriguing. The use of PB by the Gulf War personnel would probably not cause significant illness in most individuals but might cause problems in some with small stature, asthma or unique biochemistry. The two greatest limitations in identifying illness due to exposures in a theater of war are the virtual absence of exposure information and the difficulty of evaluating the health status of a self-selected group. In future conflicts, better collection of exposure information and prospective follow-up of a

statistically valid sample of the combatant population with an appropriate non-combatant control group would facilitate the identification and characterization of emerging illnesses.

RECOMMENDATIONS

A sincere and scientifically valid effort to explore and address health concerns of veterans from military conflicts is an extremely important responsibility that our government has toward its veterans. But communicating in an open, non-defensive manner with the concerned service personnel and the public about the state of knowledge and the progress of knowledge is potentially the greatest challenge facing the Department of Defense and the Veterans Administration with regard to issues of post conflict health of veterans. While the health problems from which Gulf War veterans suffer may never be completely ascribed with certainty to specific exposures that occurred during service in the Gulf, the challenge of identifying, and caring for the health of veteran's and responding to the health concerns of veterans will continue as long as there are veterans. Effective risk communication is essential to maintaining and optimized three way dialog between the veteran-active duty community, the citizenry and the responsible government branches.

SPECIFIC RECOMMENDATIONS

This author can not substantially improve on the scientific comprehensiveness of the recommendations made by the Institute of Medicine on improving the surveillance and monitoring capabilities of the DOD regarding health effects of combat service (Institute of Medicine, IOM, 1996). I do believe it is important to add that the IOM report fails to recommend a mechanism whereby the veterans, the U.S. public and active duty personnel might participate in the functioning of an ongoing system of health outcomes monitoring. Potentially the most important predictor of success of this program as judged by these constituencies is the degree to which they can claim ownership of the process. I strongly encourage that a mechanism be established to assure active participation by representatives of the U.S. public, veterans groups and active duty personnel of varied ranks and branches in the design and conduct of any program that is adopted. A mechanism should also be established to regularly communicate with all veterans providing them with ongoing information about new developments and knowledge regarding the effect of service and health.

RESEARCH IN BASIC AND APPLIED SCIENCE

Support for further research on technology for detecting environmental release and personal exposure to war gases should be a particular emphasis of the DOD. Monitors should be developed that are portable, collect and report real time information, and have data storage capabilities and are easily applied by combatants.

Research should be undertaken to develop profiles of individuals who may potentially suffer untoward effects from war gas antidotes (e.g. asthmatics, smaller individuals). Those individuals should have personal drug dosing profiles developed and confirmed by cholinesterase activity levels appropriate to the prophylactic medication taken. Routine cholinesterase testing of all personnel is probably not warranted, but the test should be available on a routine basis for evaluating ill combatants both for overdose of prophylactic medication and for evaluating war gas exposure.

A new technique described by Polhuijs (1997) potentially represents a very significant breakthrough in the detection of cholinesterase inhibited by the nerve gas sarin. Whether this technique is applicable to other nerve gases and pesticides has not been demonstrated to date. This technique should be explored and amplified if possible for application to exposure assessment of subjects potentially exposed to nerve gases and pesticides.

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POSSIBLE POTENTIATION OF PYRIDOSTIGMINE BROMIDE BY PESTICIDES

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SUMMARY

The Senate Committee on Veterans' Affairs requested a review and analysis of research on synergism or potentiation of pyridostigmine bromide (PB) toxicity by pesticides. This summary examines reports that indicate PB may become more toxic when an organism is simultaneously exposed to pesticides and other factors. This report suggests that PB has the potential to affect multiple organs and tissues, and that pesticides may synergise or potentiate the effects of PB on various organs and tissues. The author feels that knowledge of which pesticides and other chemicals potentiate PB toxicity will eventually lead to an understanding of the mechanism(s) underlying the observed interactions. When these mechanisms are understood, clearer scientific judgement, and hypothesis based models, can be used so that we may better understand whether PB may contribute to chronic illnesses. Knowledge of which biochemical systems are responsible for pesticide synergism of PB toxicity may allow avoidance of complications of PB use.

Introduction. Pyridostigmine bromide (PB) is a quaternary dimethyl carbamate that has been used to treat myasthenia gravis, a neuromuscular disorder characterized by skeletal muscle weakness (Breyer et al. 1990). Since 1986, PB has been recommended by the United States Army as a prophylactic agent for organophosphate (OP) nerve gas exposure (Dunn and Sidell 1989). Organophosphates bind irreversibly to the enzyme acetylcholinesterase (AChE) in the central (CNS) and peripheral (PNS) nervous systems and thereby prevent hydrolysis (breakdown) of the chemical neurotransmitter acetylcholine (ACh). As a result, ACh accumulates at nerve and muscle receptor sites. At muscles, this can produce excessive stimulation leading ultimately to muscle paralysis and death.

A prophylactic dose of PB (30 mg, every eight hours) binds to AChE, thereby protecting the enzyme from permanent damage by OP chemical warfare agents. Over time the PB is released and AChE activity is restored to a level needed to maintain life, providing that atropine and oxime treatments are also administered at the time of nerve gas exposure (Cook and Kolka 1992). This protocol has been shown to protect primates from the chemical warfare nerve agent Soman (von-Bredow et al. 1991, Wolfe et al. 1992).

Synergism (Potentiation). The possibility that PB could play a role in chronic illnesses increases if conditions potentiate (synergize) PB's toxicity. Such conditions might include simultaneous exposure to other chemicals/toxins such as pesticides. A simultaneous exposure to a toxin and another chemical can produce several different outcomes. These outcomes can range from no increased toxicity, an additive effect or a synergistic effect.

An additive effect is the sum of the independent effects of the chemicals. A dose of "A" may kill 5% of a population and a dose of "B" may kill 5% of a population. The effects would be additive if the same doses of "A" and "B" killed 10% of the population when given together.

Synergism, or potentiation, is an interaction that gives a more than additive effect. In a synergistic interaction, a dose of "A" that killed 5% of a population plus a dose of "B" that killed 5% of a population would kill over 10% and up to 100% of the population, when given together.

When used for nerve gas protection, PB was designed to be taken at doses that would inhibit about 30% AChE activity (Cook and Kolka 1992). Studies have shown that some pesticides increase PB's toxicity from about two-fold to ten-fold (Moss 1996) (Abou-Donia et al. 1996a) (McCain et al. 1997). Even low level potentiation of this specific PB action (AChE inhibition) might inhibit a large proportion of AChE activity, which could be fatal. Any degree of synergism of the effects of PB is therefore relevant.

PB's Effects Outside of Acetylcholinesterase Inhibition (Side Effects). It is possible to have substantial AChE inhibition by some chemicals without a resulting chronic illness. Several hundred humans were exposed to the AChE inhibitor sarin (nerve gas) at doses which caused cholinergic symptoms and substantial AChE inhibition (Sadayoshi et al. 1997), yet the authors reported that chronic delayed effects associated with poisoning by some other OPs were not present.

As mentioned above, PB's main action is acetylcholinesterase (AChE) inhibition. If PB's only action is AChE inhibition, and AChE inhibition is found unlikely to contribute to chronic symptoms, then the likelihood that PB can contribute to chronic illnesses is diminished. However, a different outcome is possible if, in addition to AChE inhibition, PB has some other specific action (side effect). If such a side effect were able to produce chronic outcomes, synergism of the side effect would increase the chronic outcomes. In this review, "side effect" means those effects which are the result of a chemical's action on a molecular target other than the presumed or known primary target for that chemical. For PB, this means effects that are the result of PB actions on a molecular target other than acetylcholinesterase. Possible side effects of PB, may be important if the side effects are potentiated by the actions of pesticides or other factors. Such a potentiation would cause the side effects to increase relative to the known cholinergic effects of PB, and might produce unexpected outcomes.

PB'S Muscarinic Side Effects. ACh causes two major types of response: nicotinic (nicotine sensitive) and muscarinic (muscarine sensitive) (Bowman and Rand 1980). PB produces more of one type of ACh induced response (muscarinic) over the other (nicotinic) (Arce et al. 1991, De-Novellis et al 1994, Muller et al. 1991). This predominantly muscarinic effect would not occur if PB's only action was acetylcholinesterase (AChE) inhibition, because blocking of AChE should elevate ACh at both nicotinic and muscarinic receptors equally. One would not expect to see one or the other effect to predominate. PB is known to directly affect cellular Ach receptors in addition to AChE inhibition (Pascuzzo et al. 1984), and PB binds to ACh muscarinic receptors (Yamamoto et al. 1996). PB therefore has one side effect of activating muscarinic receptors, in addition to its ability to inhibit AChE.

PB's Calcium Side Effects. LoPachin and Lehning (1997) stated that "Studies conducted over the past two decades indicate that calcium accumulation in injured axons has significant neuropathic implications and is a potentially unifying mechanistic event." PB induced muscle damage is probably caused by calcium leakage into cells through calcium channels, because a calcium channel blocker was able to reduce PB induced muscle damage (Meshul 1989).

PB'S Neurotoxic Esterase Side Effects. Another potential side effect target of PB is on an enzyme called neurotoxic esterase (NTE). NTE inhibition is believed to be associated with organophosphate induced delayed neuropathy (OPIDN). Some OP acetylcholinesterase inhibitors (in addition to their AChE inhibition), also inhibit NTE, and such exposure can lead to OPIDN (delayed neuropathy) in experimental animals (Lotti et al. 1993).

Many OPs inhibit both AChE and NTE (Ehrich et al. 1995). The type of toxic effect can range from purely AChE inhibition (rapid death from respiratory failure), to mostly delayed neuropathy (caused by NTE inhibition) (Lotti et al. 1993). Mixed effects can be exhibited by a single compound. Selective synergism of the NTE effect would result in selection for OPIDN symptoms over cholinergic symptoms. An example of this type of chemical manipulation was the production of OPIDN in cats by chlorpyrifos which normally causes only cholinergic symptoms (Fikes et al. 1992).

PB is a carbamate, and an AChE inhibitor. Some carbamates (in addition to AChE) inhibit NTE and therefore have the potential to cause delayed neuropathy if given chronically, or at high doses. A carbamate (PMBC) has been shown to cause delayed neuropathy in hens with repeated doses (Lotti et al. 1993). A series of other carbamates have been synthesized that also inhibit NTE (Randall et al. 1997). Carbaryl, a carbamate pesticide, has been reported to cause delayed neuropathy in a human (Dickoff et al. 1987). PB therefore has the potential to inhibit NTE and synergism of that side effect is a possible route to PB induced delayed neuropathy.

Target Organs. PB has predominately muscarinic side effects and many organs and tissues are affected by muscarinic, cholinergic chemicals such as PB (Bowman and Rand 1980). Many organs and tissues are therefore potential targets of synergised, muscarinic, side effects of PB. Examples are

the human central nervous system (CNS) which has PB sensitive, muscarinic receptors (Valcavi et al, 1991, Mazza et al. 1994, O'Keane et al. 1992). PB does not easily cross the blood-brain barrier (BBB) under "normal" conditions, however, the BBB may be more permeable under some conditions such as stress (Friedman et al. 1996). The BBB is not completely impermeable to PB, under any circumstances. PB causes CNS mediated behavioral changes in rats (Wolthuis and Vanwersch 1984), rhesus monkeys (Blick et al. 1994) and humans (Borland et al. 1985). Chronic dosing of PB resulting in a constant exposure of the BBB could result is significant amounts of PB in the CNS.

Other examples of organs and tissues that have muscarinic receptors which are potential targets of PB effects are peripheral neural tissue such as the guinea pig myenteric plexus (Mike 1994) and the rat superior cervical sympathetic ganglion (Ramcharan and Matthews 1996). There are also muscarinic receptors in the hearts of humans (Bowman and Rand 1980) and in blood vessels in the human brain (Tsukahara et al. 1989a), human skin (Stephenson and Kolka 1990), rat mesenteric vascular bed (Pinardi et al. 1992), the rabbit thoracic aorta (Tsukahara et al. 1989b) and the rat liver (Pfaffendorf and Van-Zwieten 1993). Other organs or tissues that are sensitive to muscarinic effects are the retina (Hutchins 1994) the eye's ciliary body (Farahbakhsh and Cilluffo 1994), salivary gland (Iwabuchi and Masuhara 1992), pancreas (Kato et al. 1992), tracheal smooth muscle (Thomas and Ehlert 1996), adrenal cells (Aguilar et al.1992), gut smooth muscles (Reddy et al. 1995), the spleen (Sandberg, 1994), kidney cells (Mohuczy and Garg 1992), the bladder (Kumamoto et al. 1990), gallbladder smooth muscle (von-Schrenck et al. 1993) and lung (Mak et al. 1992). Immune system cells (thymocytes and lymphocytes) are also sensitive to muscarinic chemicals (Kubera et al. 1992).

Potential Pesticide Synergists of PB Toxicity. This table is a partial list of pesticides ordered through the federal supply system for operations Desert Shield and Desert Storm (U.S. Senate 1995b). The insecticides with question marks (?) have not yet been evaluated for the ability to potentiate the toxicity of PB.

Pesticide	Insecticide Class	Synergizes PB?
permethrin	pyrethroid	yes
chlorpyrifos	organophosphate	yes
lindane	organochlorine	yes
DEET	repellant	yes
propoxur	carbamate	?
carbaryl	carbamate	?
diazinon	organophosphate	?

dichlorvos	organophosphate	?
methomyl (Fly bait)	carbamate	?
malathion	organophosphate	?
pyrethrins	pyrethroid-like	?

Of these insect control chemicals, DEET, permethrin and lindane are designed to be used in a manner that was likely to involve close personal human contact. Interest in the synergism of PB by DEET and permethrin arose as a result of disclosures to the U.S. Senate Veterans' Affairs Committee (U.S. Senate 1995a) that DEET and permethrin caused increased PB toxicity in cockroaches. Abou-Donia et al. (1996b) recently reported that the organophosphate insecticide chlorpyrifos, PB, and DEET interact synergistically.

The pesticides discussed below potentiate PB toxicity in various animals. Little is known about the specific mechanisms of these synergistic mechanisms. It will be difficult to predict whether these interactions would cause chronic health consequences until the specific mechanisms of synergistic interactions are understood.

Permethrin. Permethrin is a pyrethroid insecticide. Pyrethroids are generally thought to kill by modifying sodium channel function in nerve fibers. This leads to excessive leakage of sodium ions in nerve fibers which leads to excessive depolarization and excitation of the neurons (Matusmura 1985). Pyrethroid insecticides can also directly inhibit an enzyme that removes (pumps) calcium from inside cells of the rat brain (Alrajhi1990). Combined effects of PB (increased calcium leakage into the cells) plus permethrin (blocked calcium removal by pumps) could lead to a co-synergistic increase by these chemicals on cellular calcium. The outcome would be potentiation, by permethrin, of PB induced damage. Calcium loading, and subsequent damage, would be possible in tissues that had muscarinic (PB) receptors and permethrin sensitive calcium pumps.

PB toxicity is potentiated by permethrin in cockroaches (Moss 1996), chickens (Abou-Donia et al. 1996a), and rats (McCain et al. 1997). It is not clear whether this potentiation was caused by permethrin's actions on sodium channels, calcium pumps, or another action of permethrin. Abou-Donia et al. (1996a) suggested that, in chickens, PB prevented the breakdown of permethrin, that the permethrin action was responsible for the toxicity, and that PB was simply increasing the permethrin concentration (and therefore its effect). However, the damage and clinical signs reported in this study (Abou-Donia et al. 1996a) were similar to the results of organophosphate induced delayed neuropathy (OPIDN) and not pyrethroid poisoning. In addition to this, Buchholz et al. (1997) found that when rats were simultaneously dosed with PB and permethrin, PB caused the central nervous system tissue levels of permethrin to be lowered by 30%.

Either pyrethroid mechanism (sodium or calcium disruption) can lead to an ion imbalance within nerve cells which can lead to over-excitation and eventual direct damage to the nerves (LoPachin and Lehning (1997)). This over-excitation also leads to an inappropriate release of neurochemicals from nerves that leads to secondary physiological effects (Bowman and Rand 1980). Any of these permethrin effects have the potential to synergise the primary action of PB, or PB's known and potential side effects. The long term consequences of a simultaneous exposure to PB and permethrin cannot be predicted without knowledge of which biochemical effects are responsible for the synergism of PB toxicity.

Chlorpyrifos. Chlorpyrifos is an organophosphate (OP) insecticide which inhibits acetylcholinesterase. It can also cause organophosphate-induced delayed neuropathy (OPIDN) (Fikes et al. 1992). Because OPIDN may be related in some way to the disruption of calcium levels in cells (Abou-Donia 1993), the possibility also exists that some interaction between PB and chlorpyrifos is from the effects of both compounds on calcium maintenance in nerve cells.

PB and chlorpyrifos potentiate the toxicity of each other in chickens. A suggested reason for this was that both compounds block a detoxifying esterase enzyme that breaks down both chemicals. The neuropathy was attributed to the action of chlorpyrifos which was synergized because its breakdown was prevented by PB (Abou-Donia et al. (1996b). The authors suggested that these combined chemicals may be responsible for some manifestations of chronic illnesses in Persian Gulf War veterans. It was also suggested that the neuropathy seen was not from the effects of neurotoxic esterase (NTE) inhibition, but the symptoms reported were consistent with the effects of neurotoxic esterase (NTE) inhibition (Lotti et al. 1993, Johnson 1990).

Other Pesticides. Other pesticides may have been locally obtained. Those from the OP, carbamate and pyrethroid classes of pesticides have the potential to synergize PB toxicity because of similar modes of action. No information was found that ruled out or confirmed synergism of PB toxicity by those pesticides.

DDT is available outside of the U.S. and may have been present in the Persian Gulf. DDT does not strictly fit into the above classes, however, the mode of action of DDT is close to that of the pyrethroids in insects and vertebrates (Matusmura 1985). PB potentiates the toxicity of DDT in cockroaches and DDT may potentiate PB toxicity (Moss, unpublished data). It is therefore possible that DDT would also be a PB synergist in mammals.

Lindane. Lindane is a common organochlorine de-lousing agent. Lindane toxicity is potentiated fourteen fold in cockroaches by a sub-lethal dose of PB (Moss, unpublished data). No published research was found that dealt with synergism between PB and lindane on vertebrates. Lindane blocks inhibitory actions in the nervous system which results in over-excitation (Matusmura 1985). One of the side effects of lindane is the inhibition of a calcium ATPase, a pump that removes calcium from cells (Basavarajappa and Salimath 1990). Combined effects of PB (increased calcium leakage)

plus lindane (blocked calcium removal by pumps) would probably lead to a co-synergistic increase by these chemicals on cellular calcium. The outcome would be potentiation, by lindane, of PB induced damage. Calcium loading, and subsequent damage, would be possible in tissues that had muscarinic (PB) receptors and lindane sensitive calcium pumps. Synergistic interactions between PB and lindane in vertebrates should be investigated.

DEET (N,N-Diethyl-m-toluamide). The insect repellant DEET was developed by the U.S. Department of Agriculture in the 1950's (McCabe et al. 1954). The mechanism(s) of the repellant and toxic action(s) of DEET are still unknown. Some reports indicate that excessive doses of DEET may be toxic to humans (Clem et al. 1993, Lipscomb et al.1992, Schaefer and Peters 1992) and non-human vertebrates (Mount et al. 1991, Schoenig et al. 1993, Verschoyle et al. 1992).

DEET and PB synergize each other's toxicity in cockroaches (Moss 1996), rats (McCain et al. 1997), chickens (Abou-Donia et al. 1996a), and mice (Chaney et al. 1997a). In chickens, the synergism of DEET has been attributed to blocking of degrading enzymes (esterases) by PB so that more DEET could cross the blood-brain barrier (BBB) (Abou-Donia et al. 1996a).

We cannot understand the sub-lethal, possible long term consequences of this chemical mixture of PB and DEET without knowing DEET's mode of action. One cannot tell from current experiments which of the two (DEET or PB), is the primary toxicant, the synergist, or if both contribute to synergism and toxicity.

Moss (1996) hypothesized that DEET might have actions similar to the insect neurochemical octopamine, or the human neurochemical adrenaline. Based on that speculation, Chaney et al. (1997a,b) tested the ability of both DEET, adrenaline, and adrenergic drugs to potentiate the toxicity of PB. Chaney et al.(1997a) found that both DEET and beta-adrenergic drugs (including the native neurochemical adrenaline) synergised the toxicity of PB in mice. The synergistic interactions between PB and DEET, and PB and adrenergic drugs, were probably caused by the muscarinic side effects of PB because atropine (a muscarinic receptor blocker) eliminated the synergistic interactions (Chaney et al. 1997a). DEET's synergism of PB toxicity may be the result of adrenergic effects of DEET.

The possibility that PB will synergise the effects of adrenergic stimulation should also be investigated. In preliminary experiments (J. Moss and J. Schiffenbauer, unpublished data) it was found that PB and salbutamol (a beta-adrenergic PB synergist in mice [Chaney et al. 1997a,b]) interacted synergistically in mouse T-lymphocytes. Combined, PB and salbutamol inhibited mouse T-cell proliferation while the same drugs alone had no effect. Adrenergic drugs were originally investigated because DEET mode of action research raised the possibility that DEET had adrenergic effects. The effects on lymphocytes might range from subtle short-term effects which could be stimulation or suppression, depending on the particular type and stage of development of the cells or the effect could be outright mortality of the cells.

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A DISCUSSION OF ISSUES CONCERNING THE ROLE OF STRESS IN VETERANS' REPORTING OF SYMPTOMS FOLLOWING DEPLOYMENT TO THE GULF WAR

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THE PAC FINDING THAT STRESS IS LIKELY RELATED TO ILLNESSES IN SOME GULF WAR VETERANS

The Presidential Advisory Committee on Gulf War Veterans' Illnesses (PAC) found that (1) many Gulf War veterans have illnesses that are likely to be connected to their service in the Gulf, (2) current scientific evidence does not support the hypothesis that Gulf War veterans current illnesses were caused by a number of environmental risk factors, and (3) stress manifests in diverse ways and is likely to be an important contributing factor to the broad range of illnesses currently reported by Gulf War veterans (PAC, 1996, executive summary). Little new scientific information has emerged in the year following the report's release to question these findings with respect to the symptoms reported by large numbers of Gulf War veterans.

The PAC's conclusion regarding the likelihood that many Gulf War veterans illnesses may be stress-related may appear to be a "diagnosis by exclusion" due to their findings that the available scientific evidence did not support hypotheses that other major exposure possibilities were responsible for the broad spectrum of symptoms reported by many Gulf War veterans. However, one may argue that "stress" is the *only* potential exposure that could manifest as the wide variety of symptoms reported by a large proportion of the Gulf War veterans examined.

Unfortunately, little scientifically sound information for making this argument is to be found in the literature of studies performed on Gulf War veterans concerning the role of stress in the symptoms that they report. There is substantial confusion in the Gulf War illness literature concerning the role of stress. In part, this confusion may stem from a lack of clarity from the larger stress literature concerning the role(s) that stress plays in the occurrence of physical diseases and, more particularly, in the types of non-specific symptoms that have been reported frequently by Gulf War veterans. No doubt, some of the confusion in the Gulf War illness literature stems from authors' lack of precision in the use of language concerning stress. Some confusion probably stems from the language in the PAC final report that virtually equates "stress-related disorders" with psychological symptoms (PAC, 1996, pp. 73-79). Further, most of the available literature focuses on Post-

Traumatic Stress Disorder (PTSD, defined below), rather than the impact that sustained physical and psychological stressors may have had on veterans' health and symptom reporting.

WHAT IS STRESS?

Stress is defined as a process in which environmental demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place persons at risk for disease (Cohen, Kessler & Gordon, 1995, p.3). It is important to distinguish between components of the stress process by referring to environmental components as environmental demands, *stressors*, or events; to subjective evaluations of stressfulness of a situation as appraisals or *perceptions of stress*; and to affective, behavioral, and biological responses to stressors or appraisals as *stress responses* (paraphrased from Cohen, Kessler & Gordon, 1995, p.4).

In the general stress literature there are three broad traditions of research of assessing the role of stress in disease risk (after Cohen, Kessler & Gordon, 1995):

- *Environmental*: a focus on assessment of environmental events that are objectively associated with substantial adaptive demands.
- *Psychological*: a focus on individuals subjective evaluations of the stressfulness of a situation and their abilities to cope with those demands.
- *Biological*: a focus on the biological systems activated by psychologically and physically demanding situations.

The environmental stressors in the Gulf War environment have been addressed in several investigations. Deployed veterans reported experiencing significant levels of stress in the Persian Gulf and continued distress upon returning home (Strech et al., 1996). Potential difficulties with using combat exposure questionnaires developed for the Vietnam War veterans to measure exposure among Gulf War veterans has been discussed, and previously developed questionnaires were modified to fit better the Gulf War experiences (e.g., see Wolfe, Brown & Kelley, 1993). Even though the casualty rate was low and the combat period was brief, the threat of chemical/biological warfare agents is noteworthy, as is the use of large numbers of National Guard / Reservists, who made rapid transitions both from and back to civilian life. There seems to be little argument that Gulf War military personnel experienced exposure to substantial physical and psychological stressors in addition to actual combat: heat, crowding, long periods of idle activity but high arousal, abrupt dislocation from family and work, the threat of chemical and biological weapons attacks, etc.

Much of the psychological approach in the general stress research has focused on cognitive-emotional theories of stress, e.g., the transactional model of stress and coping (Lazarus & Folkman, 1984): Stressful experiences are construed as *transactions* between the person and the environment

in which the impact of a stressor is mediated by the person's *appraisal* of the stressor and the coping resources as his/her disposal. The person evaluates the potential threat or harm of the stressor (*primary appraisal*) as well as his/her ability to change the situation or manage negative emotional reactions (*secondary appraisal*). *Coping* efforts are aimed at problem and emotional management. The *outcomes* of the coping process are functional status and psychological well-being. Mediators of both coping efforts and outcomes include the individual's dispositional coping style and social support (paraphrased from Lerman & Glanz, 1997). These concepts and theories have been incorporated into the military's models of combat stress (e.g., Gal & Jones, 1995), stress measurement instruments used in health studies, and undoubtedly underlie the stress reaction prevention efforts of the U.S. Army's Combat Stress Control Detachments (mentioned in PAC report, 1996, pp. 26-27).

Much of the biological literature on stress in humans has focused on the measurement of biological (hormonal, physiological, and immunological) stress responses (Cohen, Kessler & Gordon, 1995). A useful review of the neurobiological and endocrinological aspects of the "stress system" and conceptual linkages to pathophysiology and medical disorders is given by Chrousos & Gold (1992). The most convincing work in the stress literature has linked stressors to hormonal responses (Baum & Grunberg, 1995), heart disease (Krantz & Falconer, 1995) and immunological changes (Herbert & Cohen, 1993; Kiecolt-Glaser JK and Glaser, 1995). Little work on biological stress responses has been reported among Gulf War veterans, although one DOD-funded project of this type is ongoing (DOD #31).

In the past, the military has (understandably) focused on two areas of research with respect to the effects of stressors. One major area of military research has been investigation of the effects of environmental and psychological stressors (e.g., sleep deprivation, heat) on military job performance, i.e., the ergonomic impact of a wide variety of physical and psychological stressors. The other focus has been medical in nature. Military medical researchers have tended to focus on the effects of combat stressors in the production of psychiatric casualties such as acute combat reactions and acute PTSD, i.e., psychiatric disease resulting from experiencing extremely psychologically stressful events (Jones, 1995). Also understandably, the Department of Veterans Affairs has focused on the treatment of chronic PTSD.

WHAT IS PTSD?

Post-traumatic stress disorder is a type of anxiety disorder in which the patient has experienced or witnessed or was confronted with an unusually traumatic event that has both of the following elements: the event involved actual or threatened death or serious injury to the patient or to others, and the patient felt intense fear, horror, or helplessness (APA, 1987). The traumatic events have to be outside the range of usual experience (e.g., combat, rape, floods, abductions, and airplane crashes, but not "ordinary" life experiences such as bereavement, divorce, and serious illness) which most people would consider extremely traumatic. The disorder is characterized by (1) repeated re-

experiencing the traumatic event (e.g., through flashbacks or repeated distressing dreams), (2) persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness, (3) persistent increased arousal not present before the event, (4) these symptoms have lasted longer than one month, and (5) these symptoms cause clinically important distress or impair work, social, or personal functioning. There is most often a delay of onset of the symptoms. Acute PTSD refers to symptoms that have lasted less than six months and chronic PTSD to symptoms lasting longer than six months. Common symptoms of PTSD patients may include sleep difficulties, exacerbation of drug/alcohol abuse, outbursts of anger, reduced social activity, and difficulty concentrating on tasks. Comorbidity with other psychiatric conditions occurs frequently. New to DSM-IV (APA, 1994) is the diagnosis category of "Acute Stress Disorder", which has similar criteria and symptoms to PTSD, although the symptoms develop immediately after the traumatic event and last for a few days to four weeks. The diagnosis of PTSD or acute stress disorder is made by a qualified psychiatrist.

A number of studies indicate that some proportion of Gulf War veterans have experienced symptoms compatible with PTSD (e.g., Perconte et al., 1993; Ross & Wonders, 1993; Sloan et al., 1995; Sutker et al., 1993). These research reports of Gulf War veterans have typically involved measurement of "symptoms of PTSD" or research case definitions derived from self-reported questionnaire scales. When others have referred to these studies (and sometimes in the reports of the studies themselves), the term "symptoms of PTSD" has often been shortened to just "PTSD". Such imprecision in language promotes confusion. Since these symptoms may not be specific to PTSD, and it is often not clear that study participants had experienced traumatic events in the Gulf War of the nature and intensity required for a diagnosis of PTSD, it is probably better to refer to the outcomes measured in these studies as simply "psychological symptoms" rather than "symptoms of PTSD".

The preoccupation with the concept of PTSD has also lead to arguments in the literature not central to investigating the role of stress in the reporting of symptoms by Gulf War veterans. For example, it has lead to a misguided attempt to show that the prevalence of PTSD among Gulf War veterans is not sufficient to support the notion that stress is the cause of all of the veterans symptoms (Haley, 1997). In fact, no study has been designed and conducted to adequately estimate the prevalence of PTSD among Gulf War veterans. Combining data from a number of studies, no matter how many, that were not designed and implemented properly to estimate the prevalence of a condition will not yield useful prevalence estimates. Also, surely the PAC's finding that stress is likely to be an important contributing factor to the broad range of illnesses currently reported by Gulf War veterans is not rebutted by a demonstration that the prevalence of one potentially stress-related outcome, "symptoms compatible with PTSD", *may not be* as high as some authors have reported.

The pre-occupation with the concept of PTSD whenever the role of stress in the symptoms of Gulf War veterans is discussed has helped to obscure the fact that virtually all differences observed between military personnel deployed to the Persian Gulf and appropriate comparison groups has been

in the self-reporting of physical and psychological symptoms. It seems more prudent to ask: What symptoms have been reported by Gulf War veterans and how might they be stress-related?

WHAT SYMPTOMS HAVE MANY GULF WAR VETERANS REPORTED?

Gulf War veterans have been observed to have a wide variety of health complaints. The most frequent primary diagnoses in the DOD's Comprehensive Clinical Evaluation Program were psychological conditions (18.4%), musculoskeletal conditions and connective tissue diseases (18.3%), symptoms, signs and ill-defined conditions (17.9%), respiratory system diseases (6.8%), digestive system diseases (6.3%), skin diseases (6.2%), and nervous system diseases (5.7%), while only 9.7% were found to be healthy. Conditions were counted differently in the VA's Registry, but a compatible pattern was observed. Patterns of symptom reporting quite compatible with the pattern of these categories have been observed in several epidemiologic studies of deployed and non-deployed Gulf War era military personnel (e.g., Iowa Study Group, 1997; Stretch et al., 1995).

It should be noted that the proportions of participants given above that were assigned each primary diagnosis illustrates the relative frequencies *within the self-referred clinical samples* and can not be generalized to the Gulf War population. Similarly, proportions of participants reporting symptoms in most of the other epidemiologic studies may be over-estimates of population prevalences, given the substantial participant self-selection in all of those studies except the Iowa study (Iowa Study Group, 1997).

In general, findings of diseases or abnormalities on objective measures of health status of Gulf era military personnel have not been observed in any of the few methodologically sound studies. One large-scale mortality study observed only an increase in unintentional illnesses among Gulf era military personnel (Kang & Bullman, 1996). Similarly, a large-scale study of morbidity (hospitalizations) among Gulf War veterans indicated no substantial excess of unexplained hospitalization among those who remained on active duty following the war (Gray et al., 1996). Other smaller studies, even among relatively self-selected or clinical groups, have shown no substantial increased abnormalities on objective neurologic (Newmark & Clayton, 1995), neuropsychological (Goldstein et al., 1995), and neuromuscular (Amato et al., 1997) tests among Gulf War veterans.

WHAT DO WE KNOW ABOUT THE REPORTING OF PHYSICAL SYMPTOMS IN GENERAL?

Self-reported symptoms are important sources of information in clinical medicine. In epidemiologic studies, they can be important outcomes when measured at the same time as other, objective measurements of health outcomes. In any case, they are subject to potential reporting bias and limitations of interpretation. The reporting of physical symptoms is moderated by a number of

factors. The following is a list of moderators of physical symptom reporting adapted from the presentation of Pennebaker (1994):

Individual factors:

- Gender: Females are more likely to report symptoms than males.
- Negative affectivity: Individuals with a history of reporting negative moods are more likely to report symptoms
- Traumatic experiences in childhood: Individuals with a history of traumatic experience in childhood are more likely to report symptoms
- Recent traumatic experiences: Individuals experiencing psychological upheavals (death of a family member, divorce, loss of job) in the past 6 months are more likely to report symptoms

Perceptual factors:

- Boring or tedious environment: amplifies bodily sensations
- Situations fraught with tension or anxiety: conflict at home or at work
- An appropriate trigger or causal attribution: new information about potentially harmful exposures

Social factors:

- Isolation at home or work: leaves more time to ponder bodily sensations, may increase anxiety, and not allow social comparison of experiences
- Social spread of the disorder: occurs along friendship lines
- Secondary gain: e.g., attention or relief from work or home responsibilities.

WHAT IS DISTINCTIVE ABOUT THE SYMPTOMS REPORTED BY GULF WAR VETERANS?

Although there have been some attempts to define "Gulf War illness" as a syndrome (e.g., Haley, Kurt & Horn, 1997), there is nothing unique about the spectrum of physical and psychological symptoms reported by a substantial proportion of returning Gulf War veterans. These symptoms are

frequently reported by healthy samples of normal individuals (Pennebaker, 1982). This constellation of symptoms is similar to that reported by many groups: patients diagnosed with somatization disorders (e.g., Robbins & Kirmayer, 1991); those meeting case definitions for Chronic Fatigue Syndrome, fibromyalgia, and Multiple Chemical Sensitivity (Buchwald & Garrity, 1994); Spanish Toxic Oil Syndrome sufferers (Lopez-Ibor et al., 1985); and a substantial proportion of populations exposed to natural and man-made disasters such as floods, earthquakes, and large fires (Bromet & Dew, 1995), radiation releases (Baum et al., 1983), and environmental chemical releases (Dayal al., 1994). Further, it appears that similar symptoms have been reported by a proportion of all combatants in the U.S. military at least since the Civil War (Hyams, Wignall & Roswell, 1996).

It seems that a viable working hypothesis about what may be similar across this wide variety of exposures and conditions is stress.

HOW CAN WE KNOW WHETHER STRESS IS CONTRIBUTING FACTOR TO SYMPTOMS REPORTED BY GULF WAR VETERANS?

Unfortunately, most of the studies of health outcomes of Gulf War veterans have not been designed or implemented in such a way that scientifically defensible inferences can be made about any likely cause (including stress) of illnesses among Gulf War veterans. The large clinical registry studies (VA Registry and CCEP) have provided valuable information about the symptoms that a large number of self-selected Gulf War veterans have, but they were not designed to allow estimation of prevalence rates of symptoms or illnesses or to make scientific inferences about the relationships between potential risk factors and illnesses. Similarly, most of the studies that have been published concerning physical and psychological symptoms of various groups of Gulf War veterans have missing or inadequate comparison groups, inadequate participant sampling methods, and poor participation rates that make scientific inferences hazardous at best (e.g., Haley et al., 1997; Haley & Kurt, 1997; Ross & Wonders, 1993).

There has been one well-designed and well-conducted population-based study of self-reported symptoms and exposures among Gulf War veterans (Iowa Study Group, 1997). Fortunately, the findings with respect to self-reported symptoms of this study are very consistent many other studies that are potentially biased. That is, in this study of 3695 Gulf War veterans, those who were deployed to the Gulf (relative to those than not deployed to the Gulf) reported more symptoms of depression (17% vs. 11%), PTSD (1.9% vs. 0.8%), chronic fatigue (1.3% vs. 0.3%), cognitive dysfunction (18.7% vs. 7.6%), bronchitis (3.7% vs. 2.7%), asthma (7.2% vs. 4.1%), fibromyalgia (19.2% vs. 9.6%), alcohol abuse (17.4% vs. 12.6%), anxiety (4.0% vs. 1.8%), and sexual discomfort (1.5% vs. 1.0%). It was also observed that the National Guard / Reserve group reported, in general, more symptoms than the regular military group. Interestingly, 83% of the regular military group and 96% of the National Guard / Reserve group reported exposure to psychological stressors.

This well-conducted study can provide an illustration of why post-event self-reported exposures are poor indicators of exposure in studies of this type. Virtually all of the self-reported symptom outcomes were each related to several of the exposure risk factors (e.g., solvents, smoke, infectious agents). Of the three outcomes reported in some detail (self-reported symptoms of depression, cognitive dysfunction, and fibromyalgia) for participants who were deployed to the Gulf, all three showed statistically significant prevalence differences between exposure risk groups based on each of at least eight different exposures. For all three of these outcomes (surprisingly) "ionizing/non-ionizing radiation" was the exposure risk having the largest prevalence difference, i.e., greater than that for solvents, lead, infectious agents, pesticides, chemical warfare agents, or pyridostigmine use. Few environmental health scientists would predict that the relationships between radiation and these three outcomes should be the strongest observed or would claim that they were biologically plausible. It seems likely that reporting biases created these relationships. (It should be noted that the authors of the paper did not emphasize or misinterpreted these findings. They are used here only to illustrate the hazards of interpreting relationships between self-reported exposures and outcomes based on self-report.)

Virtually all of the other studies of symptoms of Gulf War veterans have been conducted on samples that have participant (self-) selection bias so substantial that no valid inferences can be made from the data collected as to likely effects of environmental exposures in the Gulf War veteran population. Moreover, even when such sampling biases are well controlled, as in the Iowa study, if both the potential exposures and the outcomes (physical and psychological symptoms) are measured by means of self-report, no scientifically definitive conclusions concerning the potential relationships between these variables can be performed. Not only are both sets of measures subject to potential reporting biases, but the reporting biases will tend to be correlated, which will introduce artifactual relationships between the two sets of measurements (Cohen, Kessler & Gordon, 1995). Only in studies in which both the exposures and the outcomes are measured objectively on population-based samples with high participation rates will scientifically defensible inferences about relationships between those exposures and outcomes be possible.

HOW IS STRESS LIKELY TO AFFECT OUTCOMES IN HEALTH STUDIES?

Stress may have a negative impact on health research outcomes via at least four mechanisms:

- A direct effect on physical disease outcomes, e.g., chronic heart disease.
- A direct cause of psychopathology, e.g., PTSD or somatization disorder.
- Modulation of physiological action of infectious agents or inflammatory processes, e.g., increased susceptibility to infection.
- Modulation of the reporting of symptoms, e.g., changing the threshold for complaining about discomfort, the rating of intensity of discomfort, or considering discomfort debilitating.

There is no evidence that stressors in the Gulf War had a direct effect on physical disease outcomes. There have been diagnosed cases of PTSD that, by definition, would be evidence of the second mechanism, although the number of such diagnosed cases may not be large. Several studies of "symptoms of PTSD" might provide evidence of the second mechanism, if there the stress exposure measurements were assumed to be valid and sampling of participants were adequate. No studies of infectious agents or inflammatory processes among Gulf War veterans are available that relate those outcomes to stress exposures. No studies have been reported that address the fourth potential mechanism, and it is difficult to determine how to test it empirically. It would, at a minimum, require pre-deployment data on individuals trait negative affect (Watson & Pennebaker, 1989; Costa & McCrae, 1987). However, this mechanism is plausible, and if individuals with high negative affect volunteered to participate in the research studies than individuals with lower negative affect, it could account for many observed findings of increased reporting of a wide range of symptoms.

HOW CAN WE KNOW WHETHER STRESS IS CONTRIBUTING FACTOR TO SYMPTOMS REPORTED BY GULF WAR VETERANS?

We can't. It is not possible to test directly whether symptoms reported by Gulf War veterans are due to combinations of significant stressors that they experienced because retrospective reporting biases in assessing both exposures and symptoms cannot now be overcome.

However, we can evaluate whether data already collected on Gulf War veterans are consistent with predictions that we would make *if we assume* that the reported symptoms of many Gulf War veterans are due to exposure to significant non-toxic physical and psychological stressors. One would predict (A) that military personnel that were better inoculated against the potential effects of the physical and psychological stressors of Gulf War combat (e.g., active duty soldiers) would report fewer or less intense symptoms than those less well inoculated (e.g., reserve duty soldiers), assuming that the level of stressors experienced by the two groups were comparable. One would expect (B) that any new illness or discomfort would be more likely to be reported among those experiencing a recent significant set of stressors (i.e., Gulf War deployment) than among those not experiencing such intense stressors (e.g., deployment to Europe) or any new stressors (e.g., not deployed). One would predict (C) that military personnel experiencing conditions associated with substantial psychological distress after deployment, e.g., divorce or death in the family, would report more symptoms than those not having such experiences post-deployment. One would predict (D) that, relative to military personnel with poor social support at home, soldiers with better social support at home would report more acute symptoms in the Gulf War theatre (a weak prediction), but would report fewer symptoms after returning home (a stronger prediction). One would predict (E) that individuals having a history of childhood trauma or minor psychological trauma before deployment would report more symptoms after deployment than those without such a history. Evidence consistent with some of these predictions is available in reports of studies of Gulf War veterans, e.g., predictions A and B are supported by data in the Iowa Study (Iowa Study Group,

1997). Further, it may be possible to test some of these predictions (e.g., predictions C, D, and E) by performing additional analyses of data already collected on Gulf War veterans.

CONCLUSION

One should ***not*** read this report and come to the conclusion that it implies that the symptoms that many Gulf War military personnel have reported are simply "in their head". In truth, I do not know why so many Gulf War veterans are reporting symptoms, and the literature does not support me having a scientifically based opinion. I assume that some are experiencing conditions and disease processes that would have happened without deployment to the Gulf War. My honest conclusion is that it is quite plausible that exposure to physical and psychological stressors has exacerbated physical conditions already present in some, exacerbated psychological conditions present in some, and has decreased the threshold for complaining about ailments in some. I can think of no exposure other than the wide range of potent stressors that would have potential effects on the reporting of so many different types of symptoms.

DOD and the VA are currently funding several ongoing studies concerning stress symptoms. Unless they are population-based, the participation rates are high, and the exposures are measured objectively, they are unlikely to yield useful information about relationships between Gulf War exposures and subsequent symptoms.

The fact that most people exposed to even substantial stressors do not develop symptoms (even when a substantial number do) suggests that personal vulnerability factors may be involved. Therefore, it would seem prudent to investigate which factors might be protective for, and which factors may place individuals at risk for, experiencing symptoms following combat deployment.

RECOMMENDATIONS

Follow the PAC's recommendations on peer-review of research proposals and establishing external scientific advisory panels for large projects. The Gulf War veteran literature is loaded with papers describing studies with methodological flaws that weaken their generalizability, and in many cases their validity. Perhaps there would be fewer if all proposals had been subjected to rigorous peer review and the conduct of the studies were subjected to periodic scientific review.

Minimize the number of studies that do not have both objective exposure information and objective health outcome information. (This will probably follow if #1 is observed.) Studies relating self-reported exposures to self-reported symptoms or other measures derived from self-reports are not scientifically interpretable. Perhaps improved record-keeping of the locations of military personnel will help in developing objective exposure measures, and perhaps improved medical record-keeping of objective findings from medical tests will help provide objective health outcome measures. In

addition, improved automated techniques for acquiring health-related physiological and behavioral data might prove useful.

Fund research projects aimed at the identification of personal risk factors for the development of stress-related psychological and physical illness. For example, collect baseline data on "trait negative affect" on all individuals who may be sent into combat and perform prospective studies of how well measures of this construct predict subsequent complaints, actual disease, and use of medical services. Acquire baseline information on history of traumatic exposures, alcohol/substance abuse, etc.

Formally evaluate the effectiveness of combat stress prevention programs, e.g., the U.S. Army's Combat Stress Control Detachments, and expand them if they are found to be effective in minimizing combat and post-combat stress. What information or training prior to deployment can best "innoculate" military personnel to withstand better the whole range of combat deployment stressors? What information or procedures might improve the coping skills of military personnel post-deployment?

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AIR POLLUTANT EXPOSURE AND POTENTIAL HEALTH EFFECTS AMONG PERSIAN GULF WAR VETERANS

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SUMMARY

EXPOSURES

The Persian Gulf War was associated with increased air pollution problems in some military operations areas occupied by U.S. personnel, and in some urban areas in Kuwait and Saudi Arabia. These problems included: i) occasional increased smoke from oil well fires (and some from use of unvented kerosene heaters in enclosed spaces); ii) some short-term increases in typical combustion gases (sulfur oxides -SOx, and nitrogen oxides - NOx) from oil well fires (and NOx from increased vehicular exhaust in some areas); and iii) some increases in Volatile Organic Compounds (VOCs) related to oil well fires, increased vehicular exhaust (mostly in non-urban areas), vehicle-related activities (including sand suppression) by troops, and (it is estimated) from the use of unvented kerosene heaters.

These increases in air pollution were primarily localized to areas of military activity and areas downwind from the fires (when the plumes turned from prevailing—westerly and easterly—to southerly directions, which was not very frequent). The increases in particulate matter (PM) were incremental to existing high sand-related particulate matter (PM) found in these areas (a large proportion of which are fine particles). Some VOC and NOx emissions increased in the region after the war with a return to industrial activities and vehicular traffic in urban areas. (These exposures were in addition to those normally experienced by deployed personnel in the theater of operations, and thus included exposures to some reasonably high levels of bacillus species, pollen, fungal spores.) (A Glossary of terms is at the end of the full report in the Appendix.)

POTENTIAL HEALTH EFFECTS

It can be assumed that some acute effects occurred, based on increased levels of particulate matter and irritant gases associated with the war [e.g., diesel and turbine engine fumes, kerosene heater exhaust, artillery-related smoke, etc.]. Respiratory problems (thought not to be related to oil-well-fire pollution) were reported by U.S. troops and DOD civilian contractors, (some of whom

had pre-existing cardiopulmonary disease and may also have been smokers), and resident civilians. Sick call for respiratory complaints among U.S. military personnel comprised 19% of all sick calls, compared to a sick call rate of 7% for military personnel stationed in the States. Other potentially relevant complaints (including gastro-intestinal (GI), eye, and neuropsychological symptoms), as possibly related to air pollution exposures, were said to increase in the U.S. personnel . Information on other foreign personnel and Persian Gulf troops is limited. However, a British prospective study of 125 troops reported no significant change in lung function due to deployment in Kuwait (cf Reference 15), though this may be questioned. Further studies continue.

A Kuwaiti study reported significant increases in respiratory illnesses in the residential area of Kuwait City (cf Reference 16). In their high-risk residential populations asthma admissions to hospital did not increase immediately, but admissions for chronic obstructive pulmonary diseases (COPD: bronchitis, emphysema, bronchiectasis) did (Jan.-April 1991). They also saw increases for GI illnesses, heart disease, and psychiatric complaints. [A surveillance system was organized, and an attempt was made to create a longitudinal study of exposed and asthmatics, (by a CDC medical epidemiologist) in Kuwait city, but it appears not to have come off.] Current status of residents is not really known, though some increase in asthma was reported to a visitor. It is unlikely that the temporary increases in air pollutants due to the war and its aftermath (including the oil well fires) will have a major long-term effect in civilian, resident populations, though some individuals may have been affected. An alert system and preventive education for physicians & civilians was also attempted; implementation appears not to have occurred. These attempts should be evaluated further before new studies are suggested, designed, or implemented in the civilian population of the affected countries.

One major problem emerged, that of desert sand pneumonitis, a prolonged respiratory inflammatory process (often with some fibrosis & lung destruction), at least in U.S. and British troops. This pneumonitis is currently thought to be related to inhalation of fine sand by previously unexposed individuals. Other exposures were thought to act as adjuvants, and the pneumonitis produced was thought to affect the immune system (which will be discussed further). An autopsy study (of troops) also revealed what the pathologist called obstructive bronchitis and bronchiolitis, as well as sand particles. The sand also produced opthalmalogic (eye) problems. Thus, for some newly-exposed individuals, some long-term problems, immunologic or respiratory, may have been created. Further, it is unknown presently what effects pre-conditions (including prior treatments in the military) and other possible exposures (e.g., exposure to chemical/biological warfare [CBW] agents) may have had in foreign personnel, acutely or chronically, alone or in combination. Some on-going studies are addressing these questions. These results should be evaluated further before some of the specific studies are implemented in the U.S. personnel who served in the Persian Gulf. However, a better review of records and further evaluation of those who served is warranted.

RECOMMENDATIONS

1. Record searches in the DoD and VA Registries and in the VA healthcare system should be made to determine if deployed personnel are experiencing more respiratory problems.

2. It should be determined if there have been more respiratory diseases reported in civilians in Kuwait, and, if so, what kind of diseases.

3. An epidemiological study should be performed of respiratory and other toxic endpoints associated with specific air pollutants indicated to be of concern. It can be performed in deployed and non-deployed personnel using appropriate physiological, immunological and techniques, biomarkers of effects, and epidemiological questionnaires (including location of deployment and exposure information).

4. Further studies of absorption, inhalation and ingestion of volatile organic and similar compounds used in the Desert Shield/Desert Storm theater of operations should be performed in controlled human exposure studies, using exposures at the maximum concentrations estimated for each of these pollutants. Physiological, immunological and neurological studies should be performed in these experiments.

5. Further inhalation toxicological studies should be performed using reasonable concentrations of mixtures of fine particles/diesel fumes with specific metals, and with some of the VOCs detected in the Gulf.

6. The DVA should start a more complete registry of all Gulf-deployed personnel seen in the VA system, with follow-up of 20 years, as a valuable determinant of long-term effects. Their rates of illness and death could be compared to similar aged U.S. residents. It would be of great benefit also if clinical work-ups of these personnel were standardized and included appropriate techniques for the various long-term effects expected (e.g., respiratory diseases, neurological diseases, cancer). The recommendations stemming from the IOM and PAC panels are also worthwhile.

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MYCOPLASMA AND ILLNESS

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SUMMARY

Although during the past 30 years the clinical significance of *Mycoplasma fermentans* has been at times the center of controversy, most studies indicate that this organism should be considered normal flora of the human genital tract and throat. The available data are insufficient to conclude that *M. fermentans* is more prevalent in veterans of the Persian Gulf War than in the general population. The only reports suggesting that *M. fermentans* may be more prevalent in Gulf War veterans are the work of Drs. Garth and Nancy Nicolson. These unconfirmed reports are based on the analysis of samples from a very small number of patients and are not technically rigorous. Moreover, even if *M. fermentans* were found to be prevalent in Gulf War veterans, there is no reason to believe this organism would be responsible for the unusual symptoms referred to collectively as Gulf War Illness (GWI). Consequently, the possibility that *M. fermentans* is involved in the etiology of GWI does not warrant serious consideration.

I. *M. FERMENTANS* AND HUMAN DISEASE - A HISTORICAL PERSPECTIVE

M. fermentans has never been generally accepted as a pathogen of humans or animals. This organism is considered to be a member of the normal human flora. It is also a common contaminant of culture systems used to propagate cells in the laboratory. *M. fermentans* has been at times suspected of causing various diseases in humans and, therefore, the center of some controversy. Studies suggesting that *M. fermentans* may be a human pathogen have often proven to be irreproducible, and whether this organism is a significant cause of human disease remains unclear.

A. *M. fermentans* and arthritis

In the late 1960's it was suggested that *M. fermentans* was a cause of rheumatoid arthritis (RA) (28). This suggestion stemmed from the isolation of organisms from the synovial fluid of symptomatic patients but lost favor because of the inability of other laboratories to replicate the findings (2). As is typical for mycoplasmas, the initial report describing the isolation of *M. fermentans* from synovial fluid may well have in actuality been an example of mycoplasma contamination of the serum component of the culture medium used to recover organisms. Recently, an association between *M. fermentans* and RA has been re-investigated using ultrasensitive polymerase chain reaction (PCR) methods. Although one laboratory reported finding *M. fermentans* DNA in synovial fluid from a large number of patients with RA (26, 27), another laboratory in a very well controlled study found no *M.*

fermentans DNA in synovial fluid from either normal patients or patients with RA (11). It is generally viewed that RA is an autoimmune disease and that the involvement of *M. fermentans* is unlikely (30).

B. *M. fermentans* and cancer

In the 1960's, some clinical observations suggested an association between infections by mycoplasmas and malignancies in humans (10). Introduction of mycoplasmas to cultures of baby hamster kidney cells was reported to induce cell transformation (19). *M. fermentans* was isolated from specimens of bone marrow chiefly obtained from leukemic patients (20) and shown to induce leukemoid disease in mice (22). Studies such as these gave rise to speculation that infection by mycoplasmas may induce malignant transformation in humans. However, the prevailing notion throughout the 1970's and 1980's was that *M. fermentans* was an opportunist. The reduced resistance of the host that accompanied leukemic disease was thought to facilitate low-grade infection by the mycoplasma. Interestingly, the possibility that persistent infection by *M. fermentans* may induce malignant transformation is being re-examined in the 1990's. *M. fermentans* has been shown to induce transformation of mouse embryo cells (29, 31). Mouse cells maintained in culture are very different from a whole animal. It cannot be overly emphasized that the ability to transform mouse cells in culture may have little relevance to malignancy in humans. The possibility that mycoplasma infection might lead to malignancy in humans is very remote.

C. *M. fermentans* and AIDS

M. fermentans received little scientific attention during the late 1970's and early 1980's, but once again returned as a focus for mycoplasma research in the late 1980's. What brought *M. fermentans* to the forefront was most likely a laboratory error resulting from contamination of a cell culture system with this organism. Dr. Shih Lo reportedly isolated a novel virus from patients with AIDS in 1986 (14). The virus was obtained by isolating DNA from AIDS patients and introducing the DNA directly into a mouse cell line by a process known as transfection. The “transfected” cells produced an infectious agent, the reportedly new virus. It was later determined that the infectious agent was not a virus at all but was a mycoplasma, originally identified by Dr. Lo as a new species, *M. incognitus*, and later correctly identified as *M. fermentans* (16, 24). For a variety of reasons, mycoplasma DNA cannot possibly transfect mammalian cells. Mammalian cells and mycoplasmas possess very different factors that regulate gene expression. The mycoplasmal promoters and ribosome binding sites that serve as important signals for gene expression (transcription) and protein synthesis (translation) would not be correctly recognized by mammalian cells (8). Also, mycoplasmas do not use the typical “universal” genetic code. In most organisms including mammals, the codon TGA is a stop codon signaling the end of protein synthesis. In mycoplasmas, TGA encodes the amino acid tryptophan. When expressed in other organisms, the TGA codons in the mycoplasma genes cause the production of prematurely truncated proteins that are not functional (7). For these reasons, mammalian cells cannot use mycoplasma DNA to synthesize mycoplasma proteins, and it is not possible that “transfected” mouse cells produced mycoplasmas. The initial report describing the isolation of *M.*

fermentans (the reputed novel virus) by transfection was clearly an error. The most logical explanation was that *M. fermentans* was present as a contaminant in the cell culture system used for the transfection experiments (9).

The erroneous report of isolation of a novel virus (later identified as *M. fermentans*) from AIDS patients led investigators to examine additional patients for the presence of this infectious agent. These studies provided clear evidence that about 10% of AIDS patients have detectable levels of *M. fermentans* DNA in their blood (5). Generally, investigators assumed this finding was merely a reflection of the fact that AIDS patients carry a high load of pathogenic and opportunistic microorganisms because of their suppressed immune systems. However, some investigators, most prominently Luc Montagnier (the discoverer of HIV), began studying the possibility that *M. fermentans* may be a cofactor stimulating the development of disease (AIDS) in HIV-positive patients (4). However, various studies indicated a lack of an association between *M. fermentans* and the stage of disease in HIV patients. Also, the incidence of *M. fermentans* in blood is the same (about 10%) in both HIV-positive and HIV-negative patients (12, 13). The conclusion from these studies and others is that *M. fermentans* is most likely part of the normal flora and is not involved in the progression of disease in HIV patients. Recently, Montagnier has conceded that HIV can cause AIDS in the absence of other cofactors such as *M. fermentans* (1).

D. *M. fermentans* and respiratory disease

Although *M. fermentans* appears to be a part of the normal human flora, there have been rare cases in which patients have died from respiratory failure from what may have been an infection by *M. fermentans*. Six such cases were reported, once again from the laboratory of Dr. Lo, in 1989 and three more in 1993 (15, 18). Unfortunately, confirmatory results from other laboratories have not been reported. Whether infection by *M. fermentans* was the primary cause of death in these patients is not known. If infection by *M. fermentans* was responsible for these deaths, an explanation is lacking for why an organism that is usually associated with human normal flora would cause an invasive, acute respiratory disease in these particular patients.

Dr. Lo's laboratory also has reported that *M. fermentans* can cause fatal disease in nonhuman primates (silvered leaf monkeys) (17). These experiments were performed using only four animals and have not been repeated in any laboratory. Also, inoculation of a different primate (macaques) with high doses of *M. fermentans* has thus far failed to produce disease (A. Blanchard, unpublished data). However, macaques and monkeys are different animals, and it is conceivable that *M. fermentans* might cause disease in one species of animal and not the other. Therefore, whether *M. fermentans* is capable of causing disease in nonhuman primates is an issue that will require more experimentation if it is to be resolved.

E. *M. fermentans* and AIDS-associated nephropathy

There has been one unconfirmed report, also from Dr. Lo's laboratory, of an association between *M. fermentans* and kidney disease in AIDS patients (3). The clinical diagnosis in these patients is AIDS-associated nephropathy. If the renal complications in these patients truly result from infection by *M. fermentans*, the logical conclusion would be that this organism is an opportunist capable of causing disease in specific situations such as when the host has a weakened immune system as is in AIDS patients.

F. Summary of *M. fermentans* and human disease

M. fermentans has at times been proposed to be a human pathogen causing a variety of different diseases (arthritis, cancer, AIDS, respiratory and kidney disease). The supporting evidence for any of these possibilities is scant at best, and this organism should still be considered part of the normal human flora. However, some microbes that have been considered normal flora in the past have been shown to be pathogenic. For example, *Helicobacter pylori* was for years considered to be non-pathogenic but has recently been shown to be a cause of stomach ulcers. Also, microbes which are considered normal flora can sometimes cause significant health problems in patients who are at risk because of other factors such as a compromised immune system or tissues that have been damaged from injury or infection with other pathogens. Infectious diseases are complicated and much is not known. It is conceivable that *M. fermentans* will one day be a recognized human pathogen.

II. *M. FERMENTANS* AND GULF WAR ILLNESS

A. Prevalence of *M. fermentans*

Few studies have examined the prevalence of *M. fermentans* in the general population because the organism is presumed to be normal flora. Most studies examining prevalence have focused on patients with specific disease symptoms in an effort to determine whether an association existed between presence of the organism and disease. These studies have involved small numbers of patients and have lacked an adequate assessment of the prevalence of organisms in the general population. Obviously, different studies reach different conclusions regarding the prevalence of *M. fermentans* depending on the diagnostic methods, the patient populations, and the particular types of samples that were examined.

One recent study reported the detection of *M. fermentans* in saliva from 40% (49 of 110) of healthy adults (6). A problem with this unconfirmed study is that sensitive PCR methods were used and the negative controls (samples known not to contain *M. fermentans*) were not convincing. The experiments were designed to PCR amplify *M. fermentans* DNA from saliva, and the negative controls were PCR reactions in which no test sample (saliva) was added. The authors evidently believed their negative controls worked; they thought no PCR product was obtained. However, from a careful examination of the photograph provided in the report, it appears that negative control samples may in fact have yielded a low level of *M. fermentans* PCR product. This could only result from DNA

contamination. If the negative controls give a positive PCR product (no matter how weak), the report cannot be trusted. When very sensitive PCR methods are employed, it is critical to ensure that samples are not accidentally contaminated with DNA prior to PCR analysis. Contamination of samples that are subjected to PCR analysis is a common problem and is one reason why it is important for other laboratories to independently verify findings. Therefore, the prevalence of *M. fermentans* in saliva from healthy adults must be considered unknown until confirmation is obtained from other laboratories. Other studies are also likely flawed because of contamination of samples with *M. fermentans* DNA prior to analysis. For example, a report describing the detection of *M. fermentans* DNA from lymph nodes of AIDS patients is questionable (25).

Reports indicate that blood from about 10% of the population contains *M. fermentans* DNA, and even a higher percentage of people may contain *M. fermentans* in the throat. A study from the Institut Pasteur in France reported finding *M. fermentans* DNA in blood from 8% of HIV-negative blood donors, 15% of HIV-negative patients from a sexually transmitted disease clinic, and 6% of HIV-positive patients (13). Another study from the United Kingdom reported finding *M. fermentans* DNA in blood from 10% of HIV-positive patients and 9% of HIV-negative patients from a sexually transmitted disease clinic (12). This latter study also found *M. fermentans* DNA in throat swabs from 23% of HIV-positive patients and 20% of HIV-negative patients.

It appears that *M. fermentans* DNA is commonly detected (5-20% of patients or blood donors) by PCR analysis of blood, throat, and possibly saliva samples. PCR is the most appropriate assay for the screening large numbers of patient samples because the principle alternative, isolation of *M. fermentans* organisms by culture, is usually difficult and unreliable. However, additional studies from multiple laboratories are required to truly ascertain the prevalence of this organism.

B. Prevalence of M. fermentans in Gulf War veterans

Because most investigators consider *M. fermentans* to be normal human flora, it is surprising that an effort was made to screen samples from Gulf War veterans for the presence of this organism. Blood samples from Gulf War veterans were analyzed by a technique developed by Drs. Garth and Nancy Nicolson and referred to as Nucleoprotein Gene Tracking (NGT). NGT is a procedure in which nucleoprotein is isolated from host cells, size fractionated on polyacrylamide gels, transferred to a hybridization membrane, and probed with DNA sequences specific for *M. fermentans*. This method is similar to commonly used Southern hybridization methods, except that nucleoprotein and not purified DNA is analyzed. The stated rationale for using this method was that some DNA sequences may be specifically trapped in nucleoprotein complexes (23). The claim was that sequences complexed with nucleoprotein might be lost with conventional Southern procedures, but would be detected using the NGT method. However, the NGT system is an inappropriate diagnostic method for detection of *M. fermentans*. Even if *M. fermentans* cells were themselves present inside human cells, the mycoplasma DNA would still reside inside the mycoplasma cell and not be complexed with human nucleoprotein. A serious concern is that the efficacy of the NGT method has not been

established. The sensitivity of the method has not been established by spiking control samples with known numbers of *M. fermentans* organisms. Similarly, the specificity of the method has not been established by spiking control samples with known numbers of organisms from other species of mycoplasma.

Using the NGT method, the Nicolsons reported finding *M. fermentans* DNA in 14 of 30 patients (21). A major drawback with this report is the lack of supporting documentation. Almost no data are shown in this publication or any other report published by the Nicolsons. There is only one sample from one individual (a single lane from a single gel) in which a putative nucleoprotein complex was actually shown to react with a *M. fermentans*-specific probe. The case history of this particular individual was not described. Case history has been provided for some patients, but photographs of the Gene Tracking data for these patients are not published. In the Nicolson study, *M. fermentans* DNA was not detected in any of 21 healthy individuals used as controls. However, it is premature to conclude that the incidence of *M. fermentans* in Gulf War veterans is higher or lower than it is in the general population because the Nicolson findings have not been confirmed by other laboratories. In addition to the uncertainty of the effectiveness of the NGT method, the number of samples analyzed from Gulf War veterans is few (only 30).

As explained above, the NGT method is inappropriate for detection of *M. fermentans* in samples from Gulf War veterans because *M. fermentans* DNA resides within the mycoplasma cell and would not be present in the material assayed by this procedure, namely, host nucleoprotein. An indication of the unreliability of this technique is evidenced by the Nicolsons' finding of *M. fermentans* DNA and HIV DNA sequences present in the same nucleoprotein complexes. Some regions of the HIV genome were detected but not others, indicating that HIV in its entirety was absent. Based on this finding, the Nicolsons concluded that HIV sequences may have been inserted into *M. fermentans* by genetic engineering, with the engineered strain being released into the environment either accidentally or intentionally. The reality is that genetic engineering of *M. fermentans* is not technically feasible at the present time and certainly did not occur prior to the Gulf War. Methods for genetic engineering have been established for a few species of mycoplasma but not for *M. fermentans* (8). Also, viruses that infect humans and other animals cannot infect bacteria and mycoplasmas. One reason for this is that bacteria lack the receptors the virus needs to attach to the cell's membrane. In the case of HIV, *M. fermentans* lacks the CD4 receptor. Therefore, HIV could not enter the mycoplasma. Because the NGT method yielded an impossible result (*M. fermentans* DNA complexed with HIV DNA), none of the data obtained using this method can be trusted. Therefore, there are no valid data linking *M. fermentans* with GWI.

C. Could *M. fermentans* cause disease with symptoms similar to GWI?

Because *M. fermentans* is generally considered normal human flora, it is expected that most individuals colonized by *M. fermentans* would be healthy and have no symptoms of disease. However, as mentioned above, many microbes that are usually considered normal flora can be pathogenic if

the patient is immunocompromised. Also, there is ample evidence that synergistic interactions can occur when multiple infections are simultaneously occurring in an individual. Therefore, it is conceivable that *M. fermentans* is normal human flora and yet rarely capable of causing disease (although not demonstrated to date).

If *M. fermentans* can cause human disease, what would be the expected symptoms? Obviously, any comments in this area are speculative. Many mycoplasma species are respiratory pathogens, and as noted above, there is some evidence to suggest that *M. fermentans* may rarely be associated with respiratory disease. Also, as noted above, *M. fermentans* DNA has been reportedly detected in 20% of throat samples from HIV-positive and HIV-negative individuals. It certainly is conceivable that *M. fermentans* may cause respiratory problems and sore throats in some individuals. During an active infection, other symptoms such as fatigue and fever may be expected. These symptoms would most likely be temporary, disappearing as the infection ran its course. Several species of mycoplasma can cause arthritis in various animal hosts. It is, therefore, conceivable that *M. fermentans* could be associated with joint pain in some individuals (but, this again becomes speculative).

Specific cases involving subjects who are Gulf War veterans and have tested positive for the presence of *M. fermentans* DNA in blood samples have been reported by the Nicolsons. Most of these individuals reportedly had an array of symptoms including skin rashes, vision problems, memory loss, diarrhea, and sleep problems (21). None of these symptoms are associated with any known disease caused by any species of mycoplasma. The possibility that *M. fermentans* is responsible for these symptoms is too remote to be seriously considered based on the available scientific evidence.

FINAL COMMENTS

It is very common for individuals to come in contact with potentially dangerous microbial pathogens. These microbes are usually cleared from the body in a short period of time and result in no disease. Therefore, the mere presence of organisms, even if they are known human pathogens, is not necessarily a health concern. One factor to be considered is the site where organisms are found. For example, a particular bacterium may be of no concern if located in the intestine but a significant concern if found in the lung. Another factor is the overall health of the individual. A third factor is the virulence of the particular strain of bacteria that is found. For example, some strains of *Escherichia coli* would be considered normal flora of the human intestinal tract whereas other strains would cause potentially significant problems such as severe diarrhea. Unfortunately, virtually nothing is known about factors (if they exist) that may make one strain of mycoplasma more virulent than another. Therefore, no test is available to determine whether an individual is colonized with a particularly virulent strain. Lastly, the quantity of bacteria present in a patient is important. Often, a strain of bacteria will not cause disease unless it is present in high numbers. This is a drawback to most studies that use DNA detection to identify the presence of microbes in a host. *M. fermentans* DNA may be detected in blood or other samples from a patient, but the quantity of organisms is unknown. Because *M. fermentans* is apparently present in many healthy people, investigators are

skeptical about its pathogenic potential. However, the possibility that some strains of *M. fermentans* may be especially virulent and cause disease in susceptible individuals who happen to come in contact with a high number of such organisms cannot at this time be proven or disproven. Even an intensive effort by many laboratories could not resolve this issue in a short period of time. It would take years of research to determine whether *M. fermentans* is not simply normal flora but in fact a pathogen, but such expenditures definitely are not justified by the evidence available.

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EPIDEMIOLOGICAL STUDIES OF THE REPRODUCTIVE HEALTH OF PERSIAN GULF WAR VETERANS

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INTRODUCTION

This report reviews the epidemiologic studies that have been, and are being, conducted to assess the reproductive health of personnel that served in the 1990-91 Persian Gulf War (PGW). To this end, I have attempted to review all relevant published studies, as well as proposals, protocols, and questionnaires for ongoing studies. I have included all studies whose results were published in scientific journals or presented at scientific meetings as of December 1, 1997, as well as studies that were in progress as of that date.

With a few notable exceptions (e.g. the Oregon Health Sciences University study, and the Klemm Analysis Group Study), the completed and ongoing studies are severely limited by their incomplete exposure assessment. Because PGW veterans were potentially exposed to a wide range of chemical, biological, physical and psychological stressors, and because exposure varied with time of deployment, location, service and occupation, the deployment-nondeployment exposure classification used in most of these studies is likely to classify veterans inaccurately with respect to many exposures. As discussed in the report, these limitations are likely to result in underestimates of the risks of PGW exposure. These studies are also quite limited in their statistical power to detect increased risks of rare outcomes. Further, many of these studies are limited by their exclusion of a large proportion of PGW-exposed veterans including those no longer in active service, and National Guard/Reservists. Most of the birth defect studies, in particular, are limited by their exclusion of births in civilian hospitals, and diagnoses after the birth hospitalization.

CURRENT STUDIES

Five studies were published by December 1997 which include data on the reproductive health of PGW veterans. These are: Stretch *et al* (1995), Penman *et al* (1996), Iowa Persian Gulf Study Group (1997), Cowan *et al* (1997), and Araneta *et al* (1997). Three of these (Penman, Cowan and Araneta) examined the relationship between birth defects and PGW exposure. In connection with the Araneta publication I also discuss an additional source of case ascertainment for Goldenhar Syndrome, which is the subject of the Araneta study. The remaining two completed studies (Stretch

and the Iowa Persian Gulf Study Group) examined PGW exposure and self-reported symptoms, which included one or more reproductive symptoms or conditions.

Stretch *et al* (1995) analyze symptoms self-reported by deployed and non-deployed veterans using questionnaires mailed to 16,167 active duty and reserve personnel in the states of Hawaii and Pennsylvania. Their low response rate (31%) may be due, in part, to the fact that questionnaires were distributed to units rather than to individuals. The only reproductive outcome that was reported in this publication was "menstrual difficulties". Among active duty respondents, rates of this outcome were low and similar in deployed and non-deployed (1.7% and 1.5% respectively). Rates among reservists were higher than those reported by active duty personnel and 34% higher among deployed than non-deployed (3.1% and 2.3% respectively).

Penman *et al* (1996) evaluated birth defects and other health problems among children of veterans of two Mississippi guard units who had served in the PGW. The medical records of all (282) children of these veterans were reviewed. No concurrent control group was utilized; rates were compared to those expected from birth defects surveillance systems and previous surveys. Among 254 (90%) who were interviewed, 54 reported births that were conceived post-deployment. Medical record review was conducted to ascertain birth defects (major and minor), premature births, low birth weight and other health problems. Five birth defects (three major, two minor), five cases of low birth weight, and no stillbirths or deaths noted. No increased risks were observed compared to rates from surveillance systems. No attempt was made to characterize exposure.

The Iowa Persian Gulf Study Group (1997) estimated the prevalence of self-reported symptoms and illnesses among military personnel deployed during the PGW compared to personnel on active duty at the same time, but not deployed to the PGW (non-PGW). For this purpose, a stratified random sample was used to select a study population of 4,886 Iowa veterans. Each individual was classified as either PGW regular military, PGW National Guard/Reserve, non-PGW regular military and non-PGW National Guard/Reserve. Subjects were interviewed regarding a range of medical and psychiatric conditions. The only reproductive outcome that was reported in this publication was "symptoms of sexual discomfort". The prevalence of sexual discomfort among female partners was approximately doubled among PGW veterans compared to non-PGW veterans (5.0% vs. 2.4% for regular military and 5.4% vs. 2.1% among National Guard Reservists). Both of these comparisons were statistically significant at the 95% level.

Cowan *et al* (1997) studied the relationship between service in the PGW and the overall risk of birth defects for all US veterans. For this purpose the authors accessed live births at 135 military hospitals between 1991 and 1993. During that time, 33,998 infants were born to PGW veterans and 41,463 to non-deployed veterans at these hospitals. Birth defects, as routinely recorded on birth records, were obtained for all live births. Military records were accessed to obtain information on military service and deployment locations. Exposure was defined simply as "deployment to the PGW". While no association between PGW service and birth defects was seen for male service

members, among females there was a small, but statistically significant, increase. Using the broadest definition of congenital malformations, malformations were noted in 10.32% of births to deployed veterans versus 9.2% to nondeployed [unadjusted relative risk 1.12, 95% confidence interval (CI) (1.00 to 1.25)]. After adjustment for race, marital status and branch of service the relative risk was reduced to 1.07 (95% CI 0.94-1.22). The risk of a severe birth defect was slightly (and not significantly) lower among children of active duty women than among children of non-deployed (2.0% versus 2.1%), and both were similar to that reported by the CDC (1.9%). Six commonly occurring groups of defects were examined and none were associated with PGW exposure either in men or women. Crude (unadjusted) birth rates were significantly higher in PGW veterans than non-deployed (95.6 per 1,000 versus 93.3 per thousand). The ratio of male to female births was similar in deployed and non-deployed veterans.

The frequency of occurrence of Goldenhar Syndrome, the most severe group of anomalies to form an oculo-auricular-vertebral syndrome was estimated in deployed and non-deployed veterans by Araneta *et al* (1997). The authors ascertained cases diagnosed at birth among infants born to active-duty military personnel in military hospital using a broad screen of hospital discharge diagnoses. Potential cases were identified using 66 ICD-9-CM codes, including the general category "anomaly of skull and face bones", and selected ear anomalies. Medical record review by expert reviewers, blinded to exposure status, was used to identify definite cases of Goldenhar Syndrome among these potential cases. For all the seven cases identified, the father was the parent in the military. Five of these were offspring of PGW veterans (14.7 cases per 100,000) and two were offspring of non-deployed veterans (4.8 cases per 100,000). Thus, the relative risk was elevated (relative risk = 3.0, 95% CI 0.6 – 20.6) though not statistically significantly. The rate observed in PGW exposed was significantly higher than that reported by either the Hawaii Birth Defects Program or the Metropolitan Atlanta Congenital Defects program (4-5 per 100,000).

The Association of Birth Defect Children (ABDC) actively solicits the reporting of birth defects. As part of this activity, 18 cases of Goldenhar Syndrome were identified in veterans; 15 were deployed to the PGW. Since this registry is more likely to obtain case referrals from exposed veterans, it cannot be assumed to include a representative sample of unexposed cases.

ONGOING STUDIES

I have identified eight ongoing studies that should provide additional information on the risk of adverse reproductive outcomes among PGW veterans.

Study 3 is a comparative study of pregnancy outcomes among PGW veterans (male and female) and other active duty personnel. I could not determine whether other outcomes will be examined in this study.

Study 4 is examining differences between PGW veterans and non-deployed veterans with respect to infertility, time to conception and risk of miscarriage. In Phase I of this study a questionnaire was mailed to a random sample of 16,000 couples (8,000 couples for which one or both deployed to the PGW, and 8,000 for which neither deployed). Currently the participation rate is 46%. Phase II will consist of a telephone interview of 5,000 couples to obtain detailed information on exposures and known risk factors for infertility and miscarriage. The following four categories of married couples are included: (1) woman served in the PGW; (2) man served in the PGW; (3) woman served in the military during the PGW, but not in the Gulf area; and (4) man served in the military during the PGW, but not in the Gulf area.

Study 7 is examining the prevalence of congenital anomalies in the seven states that maintain active birth defects surveillance systems. These include all birth defects diagnosed in live births during the first year of life and in still births. This study also proposes to compare rates of preterm birth, low birth weight and still birth between PGW veterans and non-deployed veterans in the seven states. Births between 1989 and 1993 will be included in order to compare conceptions prior to, during, and after the PGW.

The California Birth Defects Monitoring Program (CBDMP) will conduct a feasibility study to determine; (1) whether Department of Defense (DOD) data on births to active duty military personnel are sufficient to allow the CBDMP to locate the medical records of these children during their first year of life; (2) whether hospital record review is possible at DOD facilities, particularly those which may be closed or have incomplete medical record information; (3) whether DOD information about structural congenital anomalies is sufficiently accurate, compared to complete hospital medical record information. This study will also determine if DOD information about the identity of inactive (separated) personnel can be linked to California vital records and CBDMP files, neither of which contains social security information.

The most unusual reproductive tract abnormality reported by PGW veterans and their spouses is the "Gulf War Vaginal Burning Syndrome". In cases of this syndrome, which can be local or systemic, severe vaginal burning and pain are reported to occur immediately on contact with the spouse's seminal fluid. A study being conducted by the University of Cincinnati has, as its first goal to determine whether this syndrome in PGW veterans is due to the same immune responses previously described for cases in the general community. Ten cases in which the husband is an exposed veteran as well as ten unaffected spouses of exposed veterans will be selected for comparison. The second goal of the study is to identify seminal plasma proteins involved in the pathogenesis of this syndrome in spouses of PGW veterans, to determine whether these are the same as the proteins identified in cases in the general population. For this purpose, five ejaculates, collected over five consecutive days will be obtained and used to isolate seminal plasma proteins from each male participant. Women will then be tested for sensitivity to these seminal proteins using skin prick tests. The third study goal is to determine the effects of PGW exposures on human seminal plasma obtained from both PGW-exposed and non-exposed males.

The Oregon Health Sciences University study will identify risk factors for Persian Gulf War Unexplained Illness (PGWUI) in veterans from the northwestern United States. For this purpose a population-based questionnaire is being mailed to a representative sample of deployed veterans within the following strata; (1) pre-combat (Desert Shield) only; (2) combat (Desert Storm) only; (3) post-combat (desert cleanup) only; and (4) two or more of these. By using a sampling strategy based on period of deployment, the role of potential risk factors such as Pyridostigmine bromide, special vaccinations and combat stress can be isolated and analyzed. Respondents to the mailed survey will provide the study population for the clinical case-control phase of the study. In this phase, the nature and pattern of exposures in cases of PGWUI and controls will be compared. A total of 250 cases and controls will be recruited for clinical testing within four months of responding to the survey.

The Department of Veterans Affairs, is conducting a three-phase study which includes a range of reproductive endpoints. In Phase I, a mailed questionnaire was sent to a random sample of 15,000 PGW veterans and a control sample of 15,000 Gulf-era veterans. To validate responses and evaluate effects of a low response rate (50%), in Phase II, 2,000 respondents among the deployed, and 2,000 among the non-deployed are being contacted by phone to obtain permission to review medical records. Further, a random sample of 8,000 non-respondents was selected to compare respondents and non-respondents. In Phase III physical examinations will be conducted on 1,000 veterans randomly selected from each group (deployed and non-deployed) as well as their family members.

The Klemm Analysis Group is conducting a two-year study comparing the health status of 10,000 women who served in the PGW with 10,000 Gulf-era military women. For this purpose a questionnaire has been developed inquiring about symptoms and conditions including adverse reproductive outcomes such as infertility, pre-term births, still births and birth defects. Detailed information on exposures before, during and after the PGW is being elicited.

RECOMMENDATIONS FOR FURTHER STUDY

Most of the studies of the reproductive health of PGW veterans conducted to date include only limited exposure assessment. The most notable exception is the Oregon Health Sciences University Study (OHSU), which can be taken as a model for this purpose. The Klemm Analysis Group questionnaire also includes a strong exposure assessment component. The birth defect studies are particularly weak in this respect, with the exception of the Iowa study, which contains a fairly extensive exposure component. Therefore, I recommend that a nested-case-control study be imbedded in Study 7, and a detailed exposure assessment be conducted, perhaps using the OHSU instrument for consistency and later comparison across studies.

The study of Arenata *et al* documents an increased risk of Goldenhar Syndrome among potentially exposed veterans. However, this increase is not statistically significant, possibly due to small numbers. Therefore, I recommend expanding this study, both to obtain additional cases and

to improve the exposure assessment. To this end I recommend first evaluating the possible additional cases of Goldenhar Syndrome which have been identified by the ABDC registry. It should be determined whether any of the 15 exposed cases identified by the ABDC includes cases that should have been identified by the Arenenta *et al* study protocol but were inadvertently missed. In other words, were all ten of the additional exposed cases identified by the ABDC ineligible for the Araneta study? Conversely, were all five exposed cases identified in Aranenta *et al* included among the ABDC cases? It is also recommended that systematic case ascertainment for Goldenhar Syndrome be expanded in both deployed and nondeployed veterans, including births to separated personnel and all births to veterans in civilian hospitals. Ascertainment throughout the first year of life, using the full medical records would be ideal. In addition, it is important to obtain detailed exposure information on all cases and a sample of controls, perhaps using the Oregon Health Sciences' questionnaire to obtain exposure information. It is also important to determine whether the cases of Goldenhar were the first live births born to veterans post-deployment. A causal relationship between this syndrome and births after one or more healthy babies seems unlikely.

The Oregon Health Sciences' University is has provided a tentative definition of Persian Gulf War Unexplained Illness (PGWUI), and is ascertaining cases of PGWUI in the Northwest. Since it is still uncertain what exposures are most relevant for reproductive illness in PGW veterans, I recommend looking for an increased incidence of reproductive abnormalities in cases of PGWUI. It is plausible that these veterans, most affected systemically by these exposures, would also exhibit more reproductive dysfunction in connection with PGW exposures. This reproductive assessment should be as complete as possible and should include serum hormone analyses on cases of PGWUI in the Northwest cohort. In addition, it would be valuable to examine semen quality in male cases. To date none of these studies has examined semen quality of veterans. Females could be asked to maintain a detailed dairy recording menstruation, frequency of intercourse and use of contraception that would allow for a precise analysis of time to conception. If daily urine samples were obtained as well, assays would provide information on early fetal loss. (See Tier II analyses, Table 6 in the full report in Appendix L).

Several sources of misclassification in the birth defect studies conducted or underway are listed above. I recommend that the magnitude of the resulting misclassification be estimated using a sample of births from Study 7. This analysis would probably have to be limited to the five states that have active birth defect surveillance for infants up to one year of age throughout the state (thus excluding California and Georgia). This could be done by obtaining as complete an ascertainment of birth defects as possible on the selected sample, and then determining how many of these birth defects would have been missed if; (1) only the birth record had been used; (2) only military hospitals had been used; (3) only active-duty personnel had been included. The degree of under reporting could then be examined as a function of severity of the defect and other covariates.

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GULF WAR REPRODUCTIVE HAZARDS

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SUMMARY

Deployed Desert Storm/Desert Shield personnel encountered a complex ambient environment which included chemical, physical and biologic hazards, as well as those of warfare itself. The complexity of this environmental matrix, the lack of record keeping for various potential exposures and the passage of time since the conflict have conspired to muddle associations between environmental exposures and any health effect—including those affecting reproduction.

Further complicating our ability to draw inferences between Gulf War service and reproductive health harm is the apparent relatively high frequency of spontaneously occurring or "background" adverse reproductive effects such as infertility, spontaneous abortions (miscarriages) and birth defects. For example, the conception rate per menstrual cycle of normal couples of reproductive age having unprotected intercourse approaches 50%. However, the viable pregnancy rate, i.e., pregnancy resulting in the birth of a viable child, is about 25% (Soules, 1985). Major fetal malformations occur in about 3% of liveborn babies, and other impairments such as low birth weight occur in many more (Kalter and Warkany, 1983).

MECHANISM OF REPRODUCTIVE TOXICITY

Although there are gender-mediated differences in chemically induced adverse reproductive outcomes, the majority of well-tested chemicals have demonstrated adverse reproductive outcomes in both males and females (Paul and Himmelstein, 1988). Adverse effects caused by reproductive toxicant exposure may be manifested at many sites in the complex pathway of reproductive function beginning with gametogeneses, and continuing through gamete interaction (fertilization), embryonic and fetal development and growth, parturition and sexual maturation of the offspring. Various biologically plausible mechanisms exist that could explain an adverse reproductive event resulting from a Gulf War exposure. These include both genetically mediated (mutation) and non-genetically-mediated events.

MALE-MEDIATED EFFECTS

The biologic plausibility of male-mediated reproductive effects has been increasingly considered and scientific evidence for such effects has grown rapidly. Wyrobek has recently reviewed the evidence for male-mediated effects manifested beyond fertilization and the multi-generational context in which reproductive health must be studied (Wyrobek, 1993).

The process of spermatogenesis, characterized by rapid cell development in the testes, is a likely target of mutagens which ordinarily interact with dividing cells. Multiple outcomes could result from such interactions including male infertility and spontaneous abortion. Besides genotoxic mechanisms, other epigenetic and non-genetic mechanisms modulate male reproductive health at the level of the normal physiologic function and the control of erection and ejaculation. Neurotoxic agents such as lead (Lancranjan, 1975) and inorganic mercury (Wharton, 1983) may thus affect sexual function.

A male contribution to spontaneous abortion can be hypothesized via a mutagenic insult to the sperm (Wyrobek, 1993), paraoccupational exposure resulting in home contamination and maternal exposure (McDiarmid and Weaver, 1993), concentration of the agent in semen (Stachel et al., 1989) and direct transmission of the agent on sperm (Yazigi et al., 1991).

REPRODUCTIVE OUTCOMES - BIOLOGIC PLAUSIBILITY

A review of the published literature, as well as reports of the Presidential Advisory Committee (PAC) and the Institute of Medicine (IOM), and minutes of the PAC hearings on Reproductive Health of Gulf War Veterans and PAC staff consultations on reproductive health was performed. These sources reflect similar over-arching opinion on the biologic plausibility of reproductive health harm, methods to ascertain potential health effects, strengths and weaknesses of existing evidence, and recommendations for the future.

While the prevalence of malformations is variously reported at about 3-5% of newborns, increasing to 10% after the first two years of life, the general public's lack of knowledge of this baseline prevalence has helped to feed fears regarding clusters of birth defects. Epidemiologic studies to date have failed to show any excess of birth defects among deployed PGW veterans, although some studies are methodologically limited and others are ongoing. Various experts testified that chasing clusters is not a good use of the public health dollar when both statistical power and exposure assessment data are so lacking. As well, very few of the major birth defects have a recognized, discrete mechanism of causation making associations between outcomes and deployment exposure difficult.

The majority of the testimony was focused on male-mediated effects due to the disproportionate number of men deployed (about 700,000) versus women (35-50,000). The most consistent consensus among experts testifying regarding mechanisms of insult resulting in reproductive health harm focused on germ cell or other damage by a direct-acting mutagenic agent. The most commonly expected outcome from such an exposure would be a spontaneous abortion due to non-viability from chromosomal aberrations or other insult in the product of conception. Other opportunities for exposure to a toxic substance included a discussion of transport of a toxicant in seminal fluid and secondary paraoccupational exposure of the woman to contaminants tracked home by the man on the clothes and shoes. These mechanisms have been suggested in other occupational/environmental settings and enjoy more relative consensus than further issues to be discussed.

From p. 160 of his testimony, Dr. Robert Brent states "There is no epidemiological information to support the suggestion that there is an increase in congenital malformations in the offspring of Desert Storm... The nature of the malformations, the types of exposures, prior studies involving human exposures to mutagenic agents and the concept of biologic plausibility make it very unlikely that there is an increase in the incidence of malformations in offspring." From p. 161, "We would not be in the present dilemma if we had a national program of congenital malformation surveillance involving every birth in the U.S."

SELF-REPORTED REPRODUCTIVE HEALTH PROBLEMS

There has been concern among PGW veterans regarding reproductive health and the questions of any adverse reproductive outcomes being deployment - related. Early versions of the CCEP and VA Gulf War Registry Examination questionnaires have been criticized for inadequate attention to these outcomes. The VA has since revised its questionnaire to include a more detailed reproductive health assessment. Dr. Susan Mather, Chief of DVA's directorate of Environmental Medicine and Public Health relates that 53,000 veterans were seen using the old questionnaire and all of these people were mailed the updated questionnaire in the last year. She estimated that about 20,000 had been returned, but were still being analyzed. She also mentioned that phase III of the Gulf War Registry Health Examination program, although looking at a small subset of the total population, will include an evaluation of spouses and children. These approaches are appropriate given the time elapsed since exposure and the attendant epidemiologic problems which arise from this.

EXPOSURE ASSESSMENT

OVERVIEW

The principal resource cited in the variety of reports reviewed regarding the exposure assessment performed for the presence of reproductive toxicants in the Gulf War theater is the U.S. General

Accounting Office (GAO) report to the chairman, Committee on Veterans Affairs U.S. Senate. This August, 1994 document addressed a number of questions regarding reproductive health concerns in the Gulf, only one of which was a charge to characterize potential reproductive toxicants present. The report identified twenty-one agents distributed among three broad hazard types - pesticides, oil fires and soil samples, and decontaminating agents. The methodology used by GAO to assemble this list was only cursorily described to include interviews and document review. As well, the lack of any non-chemical hazards identified demonstrates a limited understanding of the array of reproductive toxicants with a potential role in health risk assessment.

The classical approach in performing an exposure assessment begins with assembling candidate toxicants present in the exposure cohort's environment. This process was partially completed by the GAO. Clearly, however, the non-chemical reproductive toxicants must also be cataloged. I will attempt to at least begin that process later in this report.

After identification of hazards, the next step in an exposure assessment is the determination of exposure dose. It is this critical step that is always challenging, but in this present scenario, all but impossible to achieve. As the GAO report states, "... we did not ascertain ... exposure rates for service men and service women for these toxicants... nor perform a risk assessment of these exposures and how they might relate to possible reproductive dysfunction...". In introducing the GAO findings in testimony before the Senate Committee, Capitol Issue Area Director, Kwai-Cheng Chan stated that (referring to the twenty-one toxicants cited above), "... the concentration levels of these compounds are unknown and so are the exposure rates for specific units".

Therefore, not only are quantitative assignments of exposure dose impossible to make for a given toxicant and a given service person, or even service unit, a qualitative assignment of exposure cannot even be reliably made.

Reinforcing this observation is Dr. Grace LeMaster's testimony to the Presidential Advisory Committee staff consultation on reproductive health of Gulf War veterans, page 34: "... exposures cannot be characterized very well. It is my understanding that even vaccination records were not kept... across all these pregnancies, you have no idea what the exposures are, it's almost like three strikes against uncovering anything in this particular situation."

While the absence of environmental sampling data for the twenty-one toxicants is understandable given the deployment scenario, as may be understood for who used how much pyridostigmine, the lack of performance type records, such as vaccination data, is less comprehensible.

Also disconcerting are the anecdotal reports cited in the GAO report. This from page two of that report (referring to the hazardous exposures in the Gulf) "such as the extensive use of diesel fuel as

a sand suppressant in and around encampments, the burning of human waste with fuel oil, the presence of fuel in shower water, and the drying of sleeping bags with leaded vehicle exhaust...".

It appears that the most that is possible regarding exposure assessment will be very coarse assumptions made about certain deployed groups. Refinement as to individual toxicant exposure to an individual service person will be extremely difficult.

One potential approach to examining at least a "first cut" assessment might be that described in Dr. Linda Shortridge's testimony to the Presidential Advisory Committee (page 413). She is describing exposure assessment methodology that is being used at the University of Oregon and some of their epidemiologic studies. Regarding exposure assessment, she states, "We do, however, have an opportunity to compare and contrast groups of veterans who had separate sets of potential exposure, because they were deployed in the theater of operations for distinct identifiable periods." This might be a potentially useful and "transportable" approach to at least qualitatively refine different populations who, because of calendar time in the theater, were necessarily exposed (or not) to some different toxic substances.

EPIDEMIOLOGY OF SELF-REPORTED ENVIRONMENTAL EXPOSURES

The 1996 summary of the Department of Defense's (DOD) Comprehensive Clinical Evaluation Program (CCEP) for Persian Gulf War Veterans included data for more than 18,000 returned service members who requested a complete health evaluation. Part of the health evaluation involved questionnaire completion of a self-reported environmental history. The questions elicited information about food and water intake, and personal habits, such as smoking and exposure to passive smoke, as well as questions regarding the more uncommon chemical environmental exposures. Obviously, the circumstances of exposure, and what determines the individual service member's positive response, are variable. Frequency of exposure is also not obtained by this method. Nonetheless, it gives a sketch of what individual soldiers reported.

A similar battery of questions were included in the Department of Veterans Affairs (DVA) Persian Gulf Registry questionnaire. Responses elicited are displayed in Table 1. Of interest is the close agreement between the two sources on frequency of environmental exposures. Passive cigarette smoke, diesel exposure, oil fire smoke and tent heater fumes were most commonly reported.

The detail of the questions in both the DOD's CCEP assessment, and the DVA's assessment however, are problematic. Without adding to the number of questions either health assessment battery currently includes, more refinement of the language used in crafting questions, and some guidance given to participants about what type of exposure constitutes a clinically important "yes" to the question, could greatly enhance the value of this information.

Table 1. Frequency of Self-Reported Environmental Exposures in Gulf War Veterans (GWV)[a] and Active Duty Service Member (ADS)[b]

EXPOSURE	POSITIVE RESPONSE	
	GWV[a] (%)	ADS[b] (%)
Passive Cigarette Smoke	88.5	88
Diesel/Other Fuels/Petrochemical Fumes	90.4	88
Oil Fire Smoke	72.6	71
Tank Heater Fumes	66.6	70
Pyridostigmine Bromide	64.2	74
Personal Pesticide Use	66.7	66
Burning Trash/Feces	73.9	N/A
Skin Exposure to Fuel	73.7	N/A
ATE Non-US Food	71.3	66
Chemical Agent Resistant Paint CARC)	34.5	47
Solvent /Paints	53.6	48
Anthrax Immunization	48.7	49
Ate Contaminated Food	33.2	21
Microwaves	34.2	N/A
Bathed in Contaminated Water	28.6	20
Bathed in Non-Military Water	30.5	N/A
Bathed in/Drank Non-US Water	N/A	32
Botulism Vaccine	26.8	26
Depleted Uranium	14.2	15
Nerve Gas	14.1	61
Took Oral Meds to Prevent Malaria	N/A	22
Mustard Gas/Blistering Agent	N/A	25
Chemical Alarm	N/A	65
Witnessed Casualty	N/A	56
Witnessed SCUD Attack	N/A	54
Witnessed Actual Combat	N/A	37
Wounded in Combat	N/A	2

a = From Office of Public Health & Environmental Hazards, DVA, "Review of DVA Revised Gulf War Registry & In-Patient Treatment Files (12/97); N = 10,075

b = Percent based on participants who answered Yes or No (excludes unknown) from DOD CCEP for PGW Veterans (4/96); N = 18,075

EXPOSURE ASSESSMENT IN REPRODUCTIVE HEALTH STUDIES

Most of the studies of reproductive health of Persian Gulf War veterans, whether they be those that have been completed, or those that are ongoing, suffer from extremely weak exposure assessment. A majority of the studies use exposure assessment definitions as simple as those deployed being exposed, and those non-deployed being unexposed for controls. This is clearly inadequate.

Of the studies that are ongoing, again the very large hospital based medical record studies, such as the Cowan and Calderon studies, as well as the Araneta studies 3, 4 and 7, referred to in Dr. Swan's report, all have this significant weakness of having no address of exposure assessment, except deployment status. Of other studies that are ongoing, several do, however, address environmental exposures. These include the National Health Survey performed by the Department of Veterans Affairs; the University of Oregon's evaluation; and the planned study by the KLEMM group of 10,000 Persian Gulf War deployed women compared to non-deployed woman.

Also of interest, we should mention that the clinical study at the University of Cincinnati, looking at seminal plasma hypersensitivity reactions plans to address in a research format some of the environmental agents which may be active here by introducing some of these environmental substances in an in vitro system during the assessment of seminal plasma hypersensitivity. This type of inclusion of environmental effectors in a research protocol is something that we should like to see in future research studies.

The principal barrier to elucidating what happened or might have happened in the Gulf is the absence of exposure data. While a list of reproductive toxicants present somewhere in the Gulf theater can be drawn, its completeness and more importantly, the lack of individual or even military unit exposure information (by type of agent, concentration, duration of exposure) collude to limit what information might be drawn from the list of suspect agents. As well, the other confounding issues, not the least of which is the physiologic and psychologic impact of deployment and war making, make assigning an association of a specific exposure to a specific adverse outcome extremely difficult. None the less, there is some limited value in listing the reproductive toxicants present in the GW theater.

CANDIDATE REPRODUCTIVE TOXICANTS

The Government Accounting Office (GAO) was asked by the Senate Veterans' Affairs Committee to specify reproductive toxicants to which deployed troops were potentially exposed. In their August 1994 report to the Senate Committee, the GAO identified three broad categories of reproductive toxicants present in the Persian Gulf area: Pesticides, oil fire contaminants and decontaminating agents. The GAO was unable to supply exposure dose data nor could they determine which specific units were exposed (if at all) to each of the agents. In addition to the agents the GAO listed, other reviews have also considered exposure to pyridostigmine bromide (PB),

the prophylactic for nerve agent exposure, the various vaccine exposures, possible biologic agent exposure and mustard agent exposure. Reproductive and developmental toxicity data, as well as epidemiologic results, where available, are summarized in this section.

Frequently reported birth defects observed in the offspring of pesticide-exposed populations include neural tube defects, limb reduction defects and facial clefts. (White FM et. al., 1988; Field and Kerr 1979; Balarajan and McDowall, 1983; M. Paul, 1993). Facial clefts and neural tube defects have also been found in some, but not consistently, in studies of herbicide exposed agricultural workers and in one study of Vietnam Veterans exposed to the herbicide Agent Orange. Clarity on this issue has been hampered by lack of exposure data and small sample sizes. Limb reduction defects have been associated with residence in farming areas and agricultural work (Schwartz DA, et. al., 1986; Schwartz and Longerfo, 1988).

Maternal pesticide exposure has been found to increase the risk of facial clefts (Brogan et. al., 1980; Gordon and Shy, 1981) and for all congenital abnormalities. There has also been some disagreement in the literature regarding increased risk for spina bifida with some reporting an increase and others not seeing one (White et. al., 1988; Golding and Sladden, 1983). Also of interest, in an interview study of crop duster pilots and their sibling controls, there was no difference between groups in number of birth defects in offspring (Roan et. al, 1984).

Generally these studies have examined people with an occupational exposure to pesticides, thus presuming a relatively longer duration of exposure opportunity and higher exposure intensity than would be the case for environmentally exposed persons (pesticide users). While adverse reproductive outcome cannot be ruled out in low level exposures to pesticides (OPs) for example, such adverse effects are much less likely in the environmentally (low dose) exposed service member population than in populations occupationally exposed, such as pesticide applicators and farm workers.

OIL FIRES AND SOIL SAMPLES

A number of toxic constituents characterize oil fire exposures, with much attention given to the polycyclic aromatic hydrocarbon benzo (a) pyrene.

BENZO (A) PYRENE

Environmental characterization of Kuwait oil-well fires indicated the likely presence of numerous genotoxic contaminants. Mutagenic products of combustion including polycyclic aromatic hydrocarbons (PAH) such as benzo (a) pyrene (BAP) were a concern in performing a health risk assessment for troops deployed to Kuwait in June - September, 1991. As part of a larger health assessment of these troops, the U.S. Army Environment Hygiene Agency (USAEHA) assessed the potential for mutagenic exposure. The study employed a generic measure of mutagen exposure, sister chromatid exchange (SCE).

Frequencies of sister chromatid exchange (SCE), a measure of genotoxic exposure, were assessed in military troops deployed to Kuwait in 1991. Soldiers completed health questionnaires and had blood collected prior to, during and following deployment to Kuwait. Frequency of spontaneous SCE was determined on blood samples as a measure of mutagenic exposure. Compared to pre-deployment baseline SCE frequency means, levels obtained two months into the Kuwaiti deployment were significantly increased ($P < 0.001$) and persisted for at least one month after return to Germany. Outcome was unaffected by known personal SCE effect modifiers including smoking, age, and diet.

This study reveals a highly significant increase in mean SCE for a population of soldiers serving in Kuwait while oil-well fires burned. This increase persisted for at least one month following return to their pre-deployment assignment in Germany. Environmental exposures not due to burning oil fires may have also caused the observed increases in SCE.

The authors concluded that although a statistical increase in SCE frequency has been demonstrated in troops deployed to Kuwait, implying a genotoxic exposure, multiple candidates exist as the potential cause of this observation. At present, SCE elevations are thought to measure exposure to some genotoxic agent, but the long-term health consequences of this phenomenon have not been determined in this or other populations' exposure to genotoxicants. (McDiarmid, et al., 1995).

Another aspect of the Army's larger health risk assessment determined environmental PAH exposure which revealed low ambient levels of PAHs in the areas where soldiers were working in Kuwait. As well, measures of PAH interactions with human blood lymphocyte DNA (PAH-DNA adducts) and aromatic-DNA adducts were at their lowest levels in Kuwait compared to levels in Germany. (Poirier M. et al., in preparation).

DECONTAMINATING AGENTS

Ethylene -glycol-monomethyl ether (2-ME) and a related compound, ethylene glycolmonoethyl ether (2-EE) are widely used in industry in paints, varnishes, and thinners, and as solvents in the textile and semi-conductor industries. Health effects data in animals and humans, together with estimates of large numbers of workers potentially exposed (850,000 U.S. workers, according to NIOSH) has prompted the OSHA to begin rule-making to limit worker exposure to 0.1 ppm for 2-ME and 0.5 PPM for 2-EE for an eight hour time weighted average (TWA) exposure. This is the first OSHA rule-making specifically driven by the adverse reproductive health effects of a workplace agent.

PYRIDOSTIGMINE BROMIDE

Pyridostigmine bromide (PB) is a cholinergic agonist used in the treatment of myasthenia gravis. PB has not been demonstrated to cause increased congenital defects in rats, when exposed throughout pregnancy (Levine, 1991). A number of myasthenic women treated with PB during pregnancy have not had adverse effects in offspring attributed to the drug (Pleuche, 1979). The American Academy of Pediatrics and the WHO working group on drugs and lactation have classified pyridostigmine as compatible with breast-feeding (AAP, 1994; WHO 1988).

NON-CHEMICAL HAZARDS

A number of non-chemical hazards have been identified which may impact the reproductive health of the Persian Gulf deployed. These hazards have been recently reviewed by Agnew et al., 1991 and include heat and biohazards.

COMMENTS ON GAO RECOMMENDATIONS

Prior to making my recommendations, I would first like to comment on the recommendations that the GAO made in their testimony from August 5, 1994 regarding reproductive hazards during Operation Desert Storm. They made four recommendations at that time. The first was to guide the Secretary of Veterans' Affairs to direct a revised and expanded questionnaire and to re-register veterans who had already completed the VA registry examination in order to include reproductive health endpoints in their surveillance. I understand that this is already being done.

Secondly, they recommend that the Environmental Protection Agency, Department of Health and Human Services and DOD make additional scientific inquiry into possible synergistic effects of multiple exposures to hazards found in the Persian Gulf War. This needs to be commented upon. This would be an extremely difficult task in that even some of the individual hazards have not adequately been reviewed for reproductive and developmental toxicity, and more importantly, the exposure assessments are so poor that it is hard to see the sense that this suggestion makes. It would not be a good use of the public health dollar to start here. Rather, there are some more fundamental issues that need to be addressed by DOD that include exposure assessments and basic hazard surveillance.

The GAO's third recommendation involved establishing baseline data on various reproductive outcomes, including birth outcomes, infertility and miscarriage rates among active duty military, reservists, presumably before future conflicts. While this is a laudatory notion, it is extremely complicated, though less daunting than their follow-up suggestion which is to ascertain exposures of reproductive toxicants and some type of a warning system when the concentrations of exposure rise to what they call "dangerous levels in future conflicts". It is unclear to me how this could be done and what is a realistic way of monitoring this separate from a more basic approach which is to

use a classical industrial hygiene hierarchy of control technology which I will say more about in my recommendations.

The fourth GAO recommendation was that the DOD should develop procedures to better ensure that troops are informed of possible reproductive toxicants before future deployments and to monitor exposure levels to such hazards. Again, the hazard communication piece of this recommendation is appropriate and can certainly be built into existing training. The notion of monitoring exposure concentrations, however, is a little more naive. I think that it is more likely that exposures can be minimized by substitution and elimination of known reproductive toxicants where possible, which included the minimizing of inappropriate use of certain reproductive toxicants that have been reported by GAO and I am going to discuss further below.

RECOMMENDATIONS

1. My first recommendation would be to "stop stupid stuff". This is language used in agency parlance to mean do not keep doing things that are not defensible. Examples here are those documented in various testimony, including the use of diesel fuel as a sand suppressant and using leaded gasoline exhaust for drying sleeping bags. These presented absolutely preventable and inappropriate overexposure to reproductive toxicants in the Gulf War theater. These types of examples of easily preventable scenarios are those that need to be included in some type of a hazard communication course or program for all deployed, especially for those that are going to be supervising ground troops.

2. There is a need to develop an environmental hazardous materials training program. I would suggest here an approach similar to the National Institutes for Environmental Health Science (NIEHS) model for workers exposed to hazardous materials (hazmat). There are three or four tiers of training, the first being the most basic and the shortest, an awareness level of training, the second being more comprehensive perhaps for someone who will have some response capability, and finally a third and higher levels, perhaps a master or trainer level where there is much more detail pursued. This approach is based on a National Fire Protection Association (NFPA) standard on Professional Competence of Responders to Hazardous Materials Incidents (NFPA 472). The general purpose of the standard is to reduce the number of incidents, injuries and illnesses resulting from hazmat incidents. The scenarios reported of the inappropriate overexposure by using toxic substances in the wrong way I think are the best examples of case studies that could be used to promote the notion that there is a right way and a wrong way to handle a hazardous substance. In addition, the hazardous materials training can include some of the various health effects training and could be very similar to the hazard communication training that is required in various work places and also has been suggested by a number of experts who have testified in the various forums that were convened to examine this problem. This also would mirror recommendations for training that the GAO made as well.

3. Medical records for vaccinations and other types of health interventions must be kept. It is incomprehensible that these data were not kept during the Persian Gulf War conflict. Electronic dog tagging and other types of electronic code readers could be used and are used throughout the military to keep track of a number of less important issues and there really is no good explanation for failure to complete these types of records.

4. Documentation of pyridostigmine bromide directions given to troops needs to be made. In addition, because of the question about the potential toxicity of pyridostigmine bromide and the questionable evolution regarding safety available in the literature, it makes sense to be more careful regarding the hazard communication training that goes on for pyridostigmine bromide and to give consideration to how usage of pyridostigmine bromide could be tracked in conflict situations.

5. Serious consideration needs to be given to establishing a birth defects registry. GAO recommends looking at various outcomes in the military as a baseline, but other experts had also suggested that this really needs to be something established on a national basis. Precisely because of our inability to look at national norms, our current dilemma of trying to measure an excess of some type of untoward event in the deployed has been confounded. It is quite clear that much more of the public health dollar has been spent than would have been necessary had these types of registries been in place. The DOD could go a long way as a significant partner to HHS in contributing funding to assist in setting up this very needed national resource, and it is clear that the DOD would be a significant recipient and beneficiary of this resource in future conflicts.

6. The recent down-sizing of occupational medicine capacity in the Army at the Center for Health Promotion and Preventive Medicine (CHPPM) Aberdeen and the apparent lack of recognition the need for this expertise by the Army facility and elsewhere needs to be addressed. Many of the above cited "stupid" practices and under-recognition of toxic hazards would have been readily recognizable and easily prevented by occupational medicine personnel who possess training and expertise in toxicology and hazard prevention. The future likelihood of deployments involving ever-more complex toxic substances in weapons systems, CW counter measures, other medications and the chemical exposures of deployment itself suggest the strategic need for a substantial occupational medicine expertise.

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CARCINOGENS IN THE PERSIAN GULF CONFLICT

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INTRODUCTION

The complexity and range of environmental hazards to which deployed Desert Storm/Desert Shield personnel had exposure opportunity include members of every known hazard class: biologic agents, chemical and physical agents as well as those of warfare itself. Beyond identifying the presence of potential environmental hazards however, to assess the health risk of exposed personnel knowledge of the exposure circumstances, duration and dose of these agents is also crucial. The absence of these data severely limit the ability of public health professionals to make assessments about potential future health risk. This is generally true about most chronic health outcomes, including cancer risk, although the relatively short duration of exposure in the Gulf (months) and our current understanding of the mechanism of cancer development, make determinations of cancer risk perhaps a bit easier to elucidate than some other disease outcome.

What is known about the mechanism by which a cancer develops in humans does help clarify the likelihood for cancer development resulting from Gulf War deployment. It is generally accepted that cancer arises not from a one-time exposure, but from a series of exposures in importantly-timed multiple stages. Generally, a one time exposure is not sufficient to cause a cancer. Rather, subsequent exposures to cancer causing agents are usually required to "promote" cancer development, as are other subsequent exposures to modulating substances which may act to enhance (or mitigate) cancer "progression."

Usually these stages take place over long periods of time (years). Our knowledge of environmentally-associated cancer can be derived from occupational cohorts. Again, generally, occupational cancers-for example lung cancer in asbestos workers-develop over a prolonged duration of exposure and generally observe a "dose-response" model. That is, the most exposed workers (those with highest dose or longest duration of exposure) are the ones most likely to develop cancer. Those with a casual exposure, for a short duration, tend not to develop a malignancy. As well, cancer development usually is not observed until ten years or more after first exposure. This time lag is termed " latency". The latency issue suggests that any cancer excesses looked for now in Gulf-deployed troops would likely not be attributed to deployment because insufficient time has passed since first exposure.

There were carcinogens present in the Gulf War theater. However, this statement only addresses (and partially so) the hazard identification step—the first of four needed to assess cancer risk. The lack of exposure assessment data forces reliance on crude estimates of likely exposure.

The broad categories of toxic substances present in the Gulf which the GAO assembled for reproductive toxicity consideration can be used here to organize classes of substances which are potentially carcinogenic. Reproductive and developmental toxicants share common mechanisms with carcinogens such as an ability to interact with a cell's genetic material (genotoxicity) and interactions with a cell's communication abilities, (Vainio, 1989) which also suggest the appropriateness of using the GAO list as a starting place.

CARCINOGENS

EPIDEMIOLOGIC EVIDENCE

In examining the case for deployment-related cancer excess, we must look to epidemiologic studies. Two mortality studies of PGW veterans have been conducted (Kang and Bullman, 1995; Writer et al, 1996). Neither found excess mortality for cancer when compared to that experienced by troops deployed elsewhere during the same period.

Another study of hospitalized PGW veterans reported in preliminary findings (Coate et al, 1995) pre-war versus post-war hospitalization rates for active duty troops deployed to the PG between August 1990 and July 1991 with those of un-deployed veterans. The study found no increase of hospitalization for any cause among PGW veteran compared to control veterans.

The Cancer experience of active duty PGW service members is similar to that reflected in the epidemiologic studies. "Cancer is rare among CCEP enrollees. " (PAC Report pg.61) The types of cancer found most frequently (lymphomas, skin cancer and testicular cancer) are among the most commonly found in males of the deployed age group. These same findings are reported in the DVA experience. " Cancer also is rare among individuals in VA's Registry. There does not appear to be an unusual incidence of any specific type of cancer in this population." (PAC Report pg. 61) The same three most common cancer types seen in the CCEP population were reported in the VA registry cohort. Thus both epidemiologic evidence and registry data sources are corroborating no cancer excesses in the PGW exposed cohort.

Exposure Assessment

Epidemiology of Self-Reported Environmental Exposures

The 1996 summary of the Department of Defense's (DOD) Comprehensive Clinical Evaluation Program (CCEP) for Persian Gulf War Veterans included data for more than 18,000 returned service

members who requested a complete health evaluation. Part of the health evaluation involved questionnaire completion of a self-reported environmental history.

A similar battery of questions were included in the Department of Veterans Affairs (DVA) Persian Gulf Registry questionnaire. Responses elicited are displayed in Table 1 (Please refer to sub-section "Gulf War Reproductive Hazards" above). Of interest is the close agreement between the two sources on frequency of environmental exposures. Passive cigarette smoke, diesel exposure, oil fire smoke and tent heater fumes were most commonly reported.

The detail of the questions in both the DOD's CCEP assessment, and the DVA's assessment is problematic, however. The exposure scenario requires refinement. There are some substances for which we are more interested in chronic exposure, such as petrochemicals, diesel and particulates, and discriminating phrases could be added to those questions to enhance response value. For other substances, we are interested in only one time exposure, such as mustard agent, but even then, we are interested in whether there was skin contact or true breathing of fumes, such as in a fire or explosion.

Without adding to the number of questions either health assessment battery currently includes, more refinement of the language used in crafting questions, and some guidance given to participants about what type of exposure constitutes a clinically important "yes" to the question, could greatly enhance the value of this information. (See recommendations section).

Candidate Carcinogens

A number of carcinogens or potentially carcinogenic substances have been referred to as present in the Gulf War theater both by the IOM Committee and the PAC. I have attempted to include those substances and also have reviewed the GAO Report on Reproductive hazards to identify possible carcinogens on that list. A discussion on those agents' toxicology and evidence of carcinogenicity is displayed in an appendix. In addition, several examples of each type of hazard class will be reviewed in the text.

Pesticides

There is documentation that the DOD shipped large volumes of one OC-Lindane to the Gulf. A commonly encountered organochlorine insecticide, it is the agent used to treat head lice. (PAC p.106).

According to the National Toxicology Program (NTP), there is sufficient evidence for the carcinogenicity of various isomers of hexachlorocyclohexane (a substituent of lindane) in animals. There is inadequate human evidence for carcinogenicity however.

Sarin (O- isopropyl methylphosphonic acid)

Sarin is a chemical warfare agent which is a potentially lethal cholinesterase inhibitor. It is not listed on the IARC or NTP carcinogen list (Sidell, 1992).

Possible exposure to sarin or other Chemical Biological Warfare (CBW) agents from atmospheric dispersion after bombing and destruction of Iraqi CBW facilities have been raised in PAC reports and IOM discussions. While atomospheric models of such an exposure are controversial at best, the IOM Committee counsels ".... There is no available evidence in human or animal studies to date that exposure to nerve agents at low levels that do not produce any detectable acute clinical or physiological manifestations results in any chronic or long-term adverse health effects."[u] IOM Report page 50.

While the committee went on to make recommendations of some issues which required further research (e.g. long-term, low level exposure effects), they stated that they "..relied heavily on known toxicological and pathological effects and existing knowledge regarding short and long-term health effects of CBW agents and on findings reported from extensive DOD and DVA clinical evaluations of veterans. "As well there has been no confirmed report of clinical manifestations of acute nerve agent exposure. (IOM report pg. 50).

As has been discussed throughout this document, while a number of toxic agents were present in the GW theater, the duration and chronicity as well as intensity of exposure figure into the likelihood of adverse health effects development. This is especially true of carcinogen exposure. While some of the commonly used pesticides are animal carcinogens, they are not recognized human carcinogens and the expected exposure scenarios make cancer development unlikely.

Oil Fire and Soil Contaminants

Volatile Organic Compounds

A health study of Army personnel deployed from Germany to Kuwait in June-September 1991 included an assessment of blood concentrations of several commonly encountered volatile organic compounds (VOCs). Concern about VOC exposure from possible oil well fires suggested this component of the comprehensive health study.

Subjects were assessed in three phases, in Germany prior to deployment; several weeks after deployment in Kuwait; and upon return to Germany. Generally, there were not significant differences in findings in the three phases and VOC results were considered within the range of levels determined to be normal U.S. reference levels.

Investigators have reported only one significant elevation in VOCs among a large number of Kuwait-deployed servicemen and that was to the compound tetrachloroethylene (PCE). This compound is not usually associated with oil fires, but was also found to be higher in some firefighters in Kuwait. One suspicion is that these elevations are due to PCE exposure during weapons cleaning. (Personal Communication, D. Ashley, NCEH, CDC, Atlanta)

Particulate Matter/Air Pollutants

Dr. Lebowitz's report on air pollutants summarizes the work of a number of different investigators regarding air pollutants of different classes including particulate matter (PM), some metals and oxides of Nitrogen (NO_x) and sulfur dioxide (SO_2). He feels there is evidence for "likely acute health hazards and potential for some chronic health hazards" (Lebowitz). I believe that this broad statement is about as precise as anyone can get given the exposure assessment limitations. For some of the air pollutants Dr. Lebowitz discusses, the data are better than they are for some other toxicant classes found in the theater. I don't think the duration of exposure to the air pollutant concentrations discussed here would significantly contribute to cancer risk of the a deployed service member.

Diesel Exhaust

Diesel exhaust is a complex made up of gases and particulate produced as a waste product from diesel-powered equipment. Its major components include carbon dioxide, carbon monoxide, oxides of nitrogen and particulates. Animal studies have consistently demonstrated significant increases in lung tumors in chronically exposed (at least 24 months) animals. (IARC, 1989). Also numerous epidemiologic studies in humans demonstrate excess cancer risk (NIOSH 1988, IARC 1989). The International Agency for Research on Cancer (IARC) classifies diesel exhaust as a probable human carcinogen (Group 2A).

Benzo (a) pyrene

A number of toxic constituents characterize oil fire exposures, with much attention given to the polycyclic aromatic hydrocarbon benzo (a) pyrene. Environmental characterization of Kuwait oil-well fires indicated the likely presence of numerous genotoxic contaminants. Mutagenic products of combustion including polycyclic aromatic hydrocarbons (PAH) such as benzo (a) pyrene (BAP) were a concern in performing a health risk assessment for troops deployed to Kuwait in June - September, 1991. As part of a larger health assessment of these troops, the U.S. Army Environment Hygiene Agency (USAEHA) assessed the potential for mutagenic exposure. The study employed a generic measure of mutagen exposure, sister chromatid exchange (SCE).

Frequencies of sister chromatid exchange (SCE), a measure of genotoxic exposure, were assessed in military troops deployed to Kuwait in 1991. Soldiers completed health questionnaires and had blood collected prior to, during and following deployment to Kuwait. Compared to pre-deployment

baseline SCE frequency means, levels obtained two months into the Kuwaiti deployment were significantly increased ($P < 0.001$) and persisted for at least one month after return to Germany. Outcome was unaffected by known personal SCE effect modifiers including smoking, age, and diet.

The authors concluded that although a statistical increase in SCE frequency has been demonstrated in troops deployed to Kuwait, implying a genotoxic exposure, multiple candidates exist as the potential cause of this observation. At present, SCE elevations are thought to measure exposure to some genotoxic agent, but the long-term health consequences of this phenomenon have not been determined in this or other populations' exposure to genotoxicants. (McDiarmid, et al., 1995).

Another aspect of the Army's larger health risk assessment determined environmental PAH exposure which revealed low ambient levels of PAHs in the areas where soldiers were working in Kuwait. As well, measures of PAH interactions with human blood lymphocyte DNA (PAH-DNA adducts) and aromatic-DNA adducts were at their lowest levels in Kuwait compared to levels in Germany. (Poirier M. et al., in preparation). These results suggest that the SCE elevations observed by McDiarmid's group in this same cohort of soldiers are not due to environmental PAH exposure.

Other Toxicants

Depleted Uranium (DU)

Uranium is a naturally occurring heavy metal found in the earth's crust which is an alpha-emitting radioactive nuclide. It occurs in several isotopic combinations. Naturally occurring uranium is an isotopic mixture of U^{234} (0.005%), U^{235} (0.711%) and U^{238} (99.284%).

Depleted uranium is a byproduct of the uranium enrichment process and is a uranium compound "depleted" of U^{235} and U^{234}. Thus DU possess a radioactive activity about 60% that of naturally uranium. The Nuclear Regulatory Commission's (NRC) standard for public exposure to "man-made" sources of radiation is 100 mrem/year above background (10.CFR 20.1301).

Potential radiologic health effects from external DU exposure are thought to be small. The primary external hazards from DU are β and γ radiation. These emissions are generated by the radioactive decay of trace-levels of uranium daughter (decay) products. The radiation exposure that Army personnel receive depends on the amount of DU present, the DU component or piece of equipment in question, (kinetic energy penetrator, DU armor, etc.), the configuration (in manufacture, in storage, uploaded on a vehicle, bare penetrator, etc.) and the exposure time. The radioactive properties of DU have the greatest potential for health impacts when DU is internalized. DU can be internalized through inhalation or ingestion.

Internalized DU delivers radiation wherever it migrates in the body. Within the body, α radiation is the most important contributor to the radiation hazard posed by DU. The radiation dose to critical body organs depends on the amount of time that DU resides in the organs. When this value is known or estimated, cancer and hereditary risk estimates can be determined. (ICRP, 1977).

Health Risks from Chemical Toxicity

Because the radioactivity of DU is very low, the chemical toxicity of DU may be the more significant contributor to human health risk. Other heavy metals—such as lead, chromium, tungsten, and uranium—are also chemically toxic. The toxic properties of DU and uranium have been broadly studied (Voegtlin and Hodge, 1949, 1953; Stokinger et al., 1981; Kathren and Weber, 1988; Leggett, 1989; Diamond, 1989; Kocher,1989; Zhao and Zhao, 1990).

As has been the case throughout this report, the absence of exposure assessment data severely limit what can be said about a soldier's potential risk of a cancer outcome from a " DU" exposure. It is believed by a majority of investigators involved in following the DU-exposed soldiers from the several " friendly fire" incidents, that those soldiers with retained metal fragments are and were likely the " most exposed" because their fragment retention constitutes an " on-going" exposure of some seven year's duration. The inhalation exposures that accompanied those events are thought likely to be of greater intensity than other exposure scenarios that have been described including those involving potential exposure during rescue operations, decontamination and equipment overhaul and preparation for transport; and even more remotely exposed, in fact, more aptly environmentally rather than occupationally exposed, those with " bystander" exposure (walking by a burning Bradley, for example.) These examples constitute a model of " concentric rings" of exposure, with those involved in the friendly fire incidents in the center, those involved in the rescue, decontamination (decon) or possibly rare health surveillance activities in a intermediate circle and the more remotely, possibly one-time, environmentally exposed in the outer-most circle.

A number of human epidemiologic studies have been done in uranium miners exposed to uranium (and other potentially toxic substances in mines) over the past 30 years. Although several of these studies have found lung cancer excesses in miners, attributing these excesses to uranium has been difficult due to the presence of other hazards in the mines including radon gas, silica, other metals and possibly miners' smoking (Samet et al 1984, Gottlieb and Husen 1982; Summarized by ASTDR 1997).

These data regarding elemental uranium suggest that the radiologic cancer risk of DU exposure is likely even lower than that for elemental uranium due to the relatively lower radioactive activity of DU (0.4 uCi/gm) compared to elemental uranium (0.7 uCi/gm).

In summary, while DU is a radiologic hazard, its relatively low radiologic activity, the low likelihood of prolonged duration of exposure (except for the group with retained metal fragments),

combined with the mechanistic issues the multi-stage theory of carcinogenesis implies, suggests that a significant cancer risk from DU exposure is small. This is the opinion of both the IOM Committee and the PAC.

Mustard Agent

Mustard agent, an alkylating chemical weapon, is capable of causing covalent binding of an alkyl group (small carbon-containing groups) to genetic material (the DNA of a cell). Hence it possesses mutagenic and potentially carcinogenic activity. It is highly reactive and can cause skin and eye burns acutely. There is evidence of an increase in lung cancer from exposure. (IOM, 1993; ATSDR, 1992.)

One confirmed case of mustard agent exposure has been documented in a soldier exploring a captured bunker in Southern Iraq on March 1, 1991. It is unlikely that there was widespread or significant exposure to mustard agent in the absence of other reports of acute effects.

Aflatoxin

Aflatoxin, a naturally occurring toxin elaborated from mold growing on some stored grains, peanuts or other food stuff under certain storage conditions, is raised as a potential environmental carcinogen. There is epidemiologic evidence that aflatoxin ingestion is associated with an excess of liver cancer and that liver cancer incidence is higher in geographic areas where there is aflatoxin excess (e.g. China) (Wogan, 1992). However, the exposure scenario and evidence which could make this toxicant a plausible candidate for widespread concern is absent.

Increased rates of liver cancer could result decades following low-level exposure, although available evidence reviewed by the committee does not indicate such exposures occurred during the Gulf War." PAC Report p. 112.

RESEARCH REGARDING CANCER

There is little government sponsored ongoing research activity, specifically regarding cancer risk. Given the summary of biologic plausibility and exposure scenarios recounted thus far, this lack of activity is not particularly inappropriate. If there is a cancer excess to be documented in deployed troops, we know that the latency between first exposure, and onset of disease, is usually many years (normally at least ten), and therefore any excesses are still to be found in the future.

There are a number of applied (rather than human epidemiologic) studies ongoing which do relate to potential cancer risk. These include the study titled "Biomarkers of Susceptibility and Polycyclic Aromatic Hydrocarbon (PAH) Exposure", part of the U.S. Army Kuwaiti oil fire health risk assessment (project # HHS-3). The depleted uranium (DU) basic studies, including an animal

study of imbedded DU metal fragments (project #DOD-7A) being done at the Armed Forces Radiobiology Research Institute (AFRRI) in Bethesda, and an inhalation toxicology study of DU fragment carcinogenicity (project #DOD-7B) performed at the Inhalation Toxicology Laboratory of the Department of Energy in Albuquerque are also ongoing.

Some studies already completed have helped inform this report. For example, the U.S. Army Kuwaiti oil fire health risk assessment results (DOD-16; DOD-18) have been reported in this document in the section discussing polycyclic aromatic hydrocarbons and volatile organic compounds.

Although listed as environmental toxicology studies, several of these projects may have important input regarding exposure assessment for carcinogens. These include the characterization of emissions from tent heaters (project #DOD-34) ongoing at the U.S. DOE Laboratory at Albuquerque, the Persian Gulf Veterans Health Tracking System (project #DOD-19) at the Center for Health Promotion and Preventive Medicine (CHPPM) at Aberdeen, and the Retrospective Verification of Mustard Gas Exposure Project (VA-47) at the Louisville VAMC, may contribute. Although this study's aim is to correlate mustard gas exposure to reproductive risk, its applicability to cancer risk is also clear.

Another basic research study with a non-cancer focus, but with potential application to the cancer question, is a project titled "DNA Damage From Chemical Agents, and its Repair" (project #VA-6D) at the Portland VAMC. Here the focus is on nervous system insult from mustard exposure. However, some of the measures of DNA- mustard interactions (DNA adducts) may be applicable to cancer (and reproductive hazard) questions.

Epidemiologic studies that are examining the cancer question include an ongoing mortality study of veterans (project VA-1) and a completed study of U.S. military personnel (project #DOD-15). Also of interest is an ongoing Boston VAMC study of Gulf War and Vietnam veterans cancer incidence (project VA-4C). This study involves linking rosters of Gulf War veterans to state cancer registries in the New England area. These record linkage studies tend not to focus on specific environmental exposures, but would look as Persian Gulf War service as the exposure, and compare results to non-Persian Gulf War deployed veterans. This is a reasonable way to do surveillance for the unlikely, but possible cancer excesses which might arise from Persian Gulf War deployment.

RECOMMENDATIONS

1. The inappropriate use and application of toxic substances (diesel fuel used as a sand suppressant) needs to be identified and stopped. Training in hazardous materials handling and

common sense handling of these substances needs to be implemented. There is a need to develop an environmental hazardous materials training program. I would suggest here an approach similar to the National Institutes for Environmental Health Science (NIEHS) model for workers exposed to hazardous materials (hazmat). There are three or four tiers of training, the first being the most basic and the shortest, an awareness level of training, the second being more comprehensive perhaps for someone who will have some response capability, and finally a third and higher levels, perhaps a master or trainer level where there is much more detail pursued. This approach is based on a National Fire Protection Association (NFPA) standard on Professional Competence of Responders to Hazardous Materials Incidents (NFPA 472). The general purpose of the standard is to reduce the number of incidents, injuries and illnesses resulting from hazmat incidents. The scenarios reported of the inappropriate overexposure by using toxic substances in the wrong way I think are the best examples of case studies that could be used to promote the notion that there is a right way and a wrong way to handle a hazardous substance. In addition, the hazardous materials training can include some of the various health effects training and could be very similar to the hazard communication training that is required in various work places and also has been suggested by a number of experts who have testified in the various forums that were convened to examine this problem. This also would mirror recommendations for training that the GAO made as well. The NIEHS model of tiered hazmat training is suggested.

2. Exposure assessment questions on self-reported clinical evaluations of DVA and DOD require refinement. While a fairly complete "laundry list" of potential exposures is elicited, information regarding crucial aspects of the exposure are lost because of the way the question is worded. Most of the questions from both sources are worded like: "While in the Persian Gulf, do you believe you were exposed to any of the following?" It is not clear to the service member what constitutes a positive answer. For example, exposure to diesel fumes, the most common affirmative response reported (90% of veterans and 88% of active duty service members) could likely have been elicited by anyone riding in a vehicle. More discriminating information could have been elicited, such as attempting to determine more intense exposure, that is occupational diesel exposure arising from, say, assignment to vehicle maintenance or transport. This compared to an "environmental" exposure opportunity of any vehicle rider, which is what is suggested by an open ended question like "have you ever been exposed?". This simple discrimination would lend some semi-quantitative information about exposure intensity. The DVA questionnaire gives a good example of a simple improvement in questioning, which refines the information elicited. When asking about diesel or petrochemical exposure, it asked about skin contact. While it is understood that only so much detail can be captured, some simple refinement of questions could enhance the value of the information obtained without increasing the number of questions. Tightening up the overall summary questions from "were you ever" to "were you, as part of your job duties working with"; or "did you have skin exposure to..."; or "other than bystander exposure, did you work with or regularly (define time frequency appropriate to the substance in question) handle substance X?"

3. As the PAC report suggested, surveillance for cancer development can be planned for and implemented although care to refine exposure assessment questions for epidemiologic tools needs to be brought to the process. Similar suggestions regarding exposure assessment as discussed in #2 above also apply here. PAC Rec. pg. 126. "DOD & VA should perform long-term mortality studies of GW veterans appropriate for investigating cancer rates in the Gulf War veteran population in coming decades."

4. Future surveillance of the DU-exposed "friendly fire" cohort is required. This group is perhaps the only undisputed carcinogen-exposed cohort identified from the deployment. Although we are heartened by good health outcomes up to now and the relatively lower radioactive intensity of DU compared to natural uranium, the exposure circumstances of retained metal fragments have not been previously encountered and represents an on-going exposure. We are obliged to follow them forward.

5. Some mechanism should be crafted to allow investigations working on potentially over-lapping areas but in separate disciplines to communicate. For example, work on a method to verify mustard gas exposure being pursued at the Louisville VAMC should be discussed with investigators at the Portland VAMC, also looking at mustard-DNA interactions but from a neurotoxicity vantage point. One group's work may inform the other's.

6. The recent down-sizing of occupational medicine capacity in the Army and the apparent lack of recognition of the need for this expertise by the Army at the Center for Health Promotion and Preventive Medicine (CHPPM) Aberdeen and elsewhere needs to be addressed. Many of the above cited "stupid" practices and under-recognition of toxic hazards would have been readily recognizable and easily prevented by occupational medicine personnel who possess training and expertise in toxicology and hazard prevention. The future likelihood of deployments involving ever-more complex toxic substances in weapons systems, CW counter measures, other medications and the chemical exposures of deployment itself suggest the strategic need for a substantial occupational medicine expertise.

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